EXHIBIT A

US005095030A

United States Patent [19]

Levy et al.

[11] Patent Number: 5,095,030

[45] Date of Patent: * Mar. 10, 1992

[54] WAVELENGTH-SPECIFIC CYTOTOXIC AGENTS

[75] Inventors: Julia G. Levy; David Dolphin; Jack K. Chow; Ethan Sternberg, all of Vancouver, Canada

[73] Assignee: University of British Columbia, Vancouver, Canada

[*] Notice: The portion of the term of this patent subsequent to Apr. 24, 2007 has been disclaimed.

[21] Appl. No.: 414,201

[22] Filed: Sep. 28, 1989

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 221,161, Jul. 19, 1988, Pat. No. 4,920,143, which is a continuation-in-part of Ser. No. 41,680, Apr. 23, 1987, Pat. No. 4,883,790, which is a continuation-in-part of Ser. No. 5,204, Jan. 20, 1987, abandoned.

[30] Foreign Application Priority Data

Jan. 19, 1988	[CA]	Canada	556875
Jan. 19, 1988	[EP]	European Pat. Off.	8830 0409.5
Jan. 20, 1988	[JP]	Japan	63-11847
Jul. 19, 1988	[JP]	Japan	63-181442

[51] Int. Cl.[5] A61K 31/40; C07D 487/22

[52] U.S. Cl. 514/410; 540/145

[58] Field of Search 514/410; 540/145

[56] References Cited

U.S. PATENT DOCUMENTS

2,951,600	9/1960	Sharp	204/162
4,268,947	5/1981	Hile	29/451
4,485,806	12/1984	Akers	128/1 R
4,500,507	2/1985	Wong	424/1.1
4,512,762	4/1985	Spears	604/21
4,577,636	3/1986	Spears	128/654
4,649,151	3/1987	Dougherty et al.	514/410
4,675,338	6/1987	Bommer et al.	514/410
4,727,027	2/1988	Wiesenhahn et al.	435/173
4,748,120	5/1988	Wiesenhahn et al.	435/173
4,753,958	5/1988	Weinstein et al.	514/410
4,883,790	11/1989	Levy et al.	540/145
4,920,143	4/1990	Levy et al.	514/410

FOREIGN PATENT DOCUMENTS

0175617 3/1986 European Pat. Off. .

OTHER PUBLICATIONS

Dougherty et al., "Porphyrin Photosensitization", Kessel et al., editors, (1983), Plenum Press, pp. 3–13.
Gregorie et al., *Ann. Surg.*, (1968), 167:827–829.
Diamond et al., *Lancet*, (1972), 2:1175–1177.
Dougherty et al., *Cancer Research*, (1978), 38:2628–2635.
Dougherty et al., "The Science of Photo Medicine", (1982), Regan & Parish, editors, pp. 625–638.
Dougherty et al., "Cancer: Principles and Practice of Oncology", (1982), Devita et al., editor, pp. 1836–1844.
Weishaupt et al., *Cancer Research*, (1976), 36:2326–2329.
Dougherty et al., "Porphyrin Localization and Treatment of Tumors", (1984), pp. 301–314.
Dougherty, *CRC Critical Reviews in Oncology/-Hematology*, (1984), 2(2):83–116.

(List continued on next page.)

Primary Examiner—Richard L. Raymond
Attorney, Agent, or Firm—Irell & Manella

[57] ABSTRACT

A group of hydro-monobenzoporphyrins "green porphyrins" (Gp) having absorption maxima in the range of 670–780 nanometers is useful in treating disorders or conditions which are subject to hematoporphyrin derivative (HPD) treatment in the presence of light, or in treating virus, cells and tissues generally to destroy unwanted targets. The use of the Gp of the invention permits the irradiation to use wavelengths other than those absorbed by blood. The Gp of the invention may also be conjugated to ligands specific for receptor or to specific immunoglobulins or fragments thereof to target specific tissues or cells for the radiation treatment. Use of these materials permits lower levels of drug to be used, thus preventing side reactions which might destroy normal tissues.

12 Claims, 4 Drawing Sheets

OTHER PUBLICATIONS

Mew et al., *J. Immunol.*, (1983), 130(3):1473–1477.
Mew et al., *Cancer Research*, (1985), 45:4380–4386.
Oseroff et al., *Proc. Natl. Acad. Sci. USA*, (1986), 83:8744–8748.
Richter et al., *J. Natl. Cancer Inst.*, (1987), 79(6):1327–1332.
Morgan et al., *J. Chem. Soc. Chem. Commun.*, (1984), pp. 1047–1048.
Pangka et al., *J. Organic Chem.*, (1986), 51:1094–1100.
Steele et al., *Cancer Immunol. Immunotherapy*, (1988), 26(2):125–131.
Wat et al., *Prog. Clin. Biol. Res.*, (1984), 170:351–359.
Levy et al., *Lasers Surg. Meth.*, (1985), 5(2):141.

FIG. 1-1

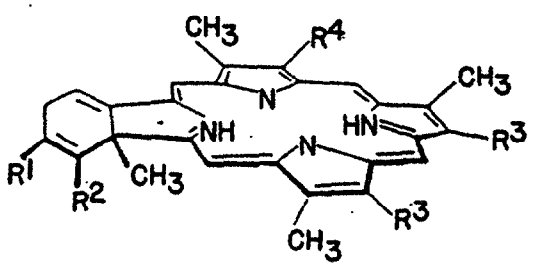


FIG. 1-2

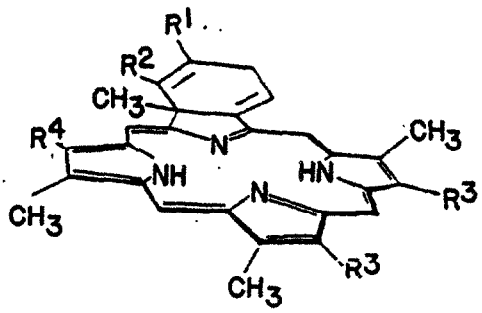


FIG. 1-3

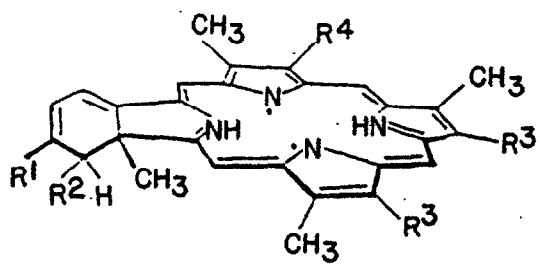


FIG. 1-4

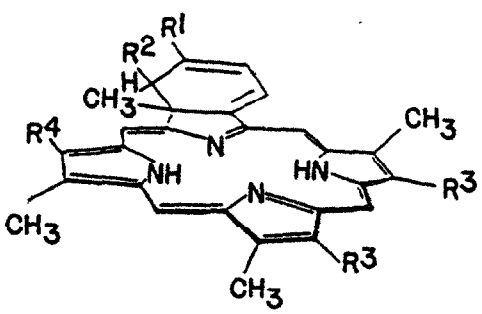


FIG. 1-5

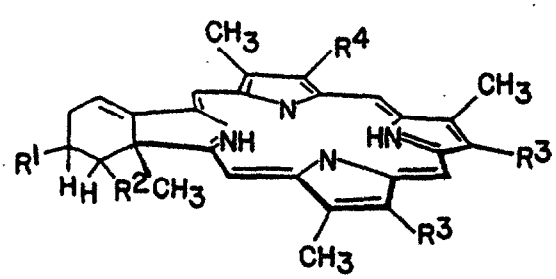
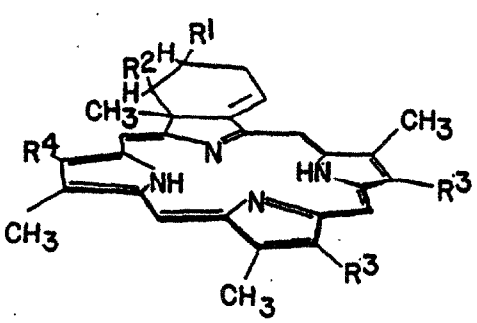
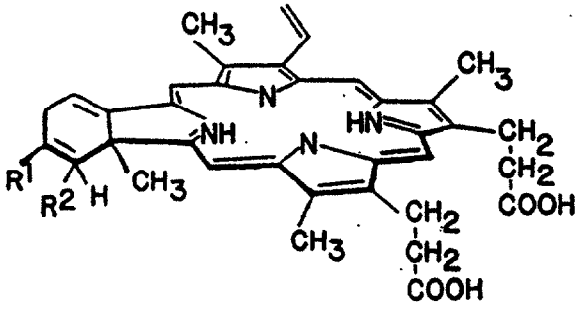


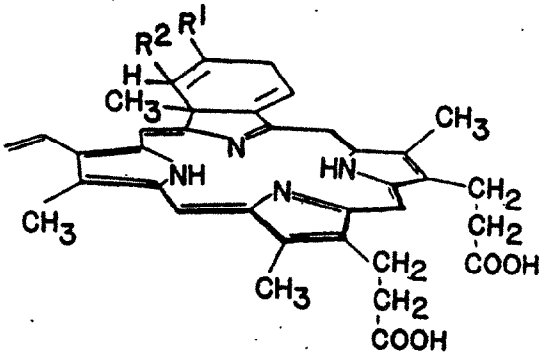
FIG. 1-6





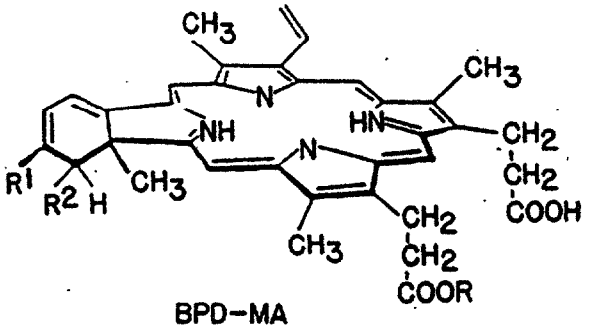
BPD-DA

FIG. 2-1



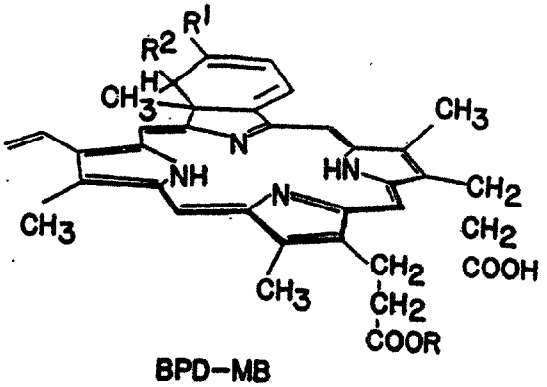
BPD-DB

FIG. 2-2



BPD-MA

FIG. 2-3



BPD-MB

FIG. 2-4

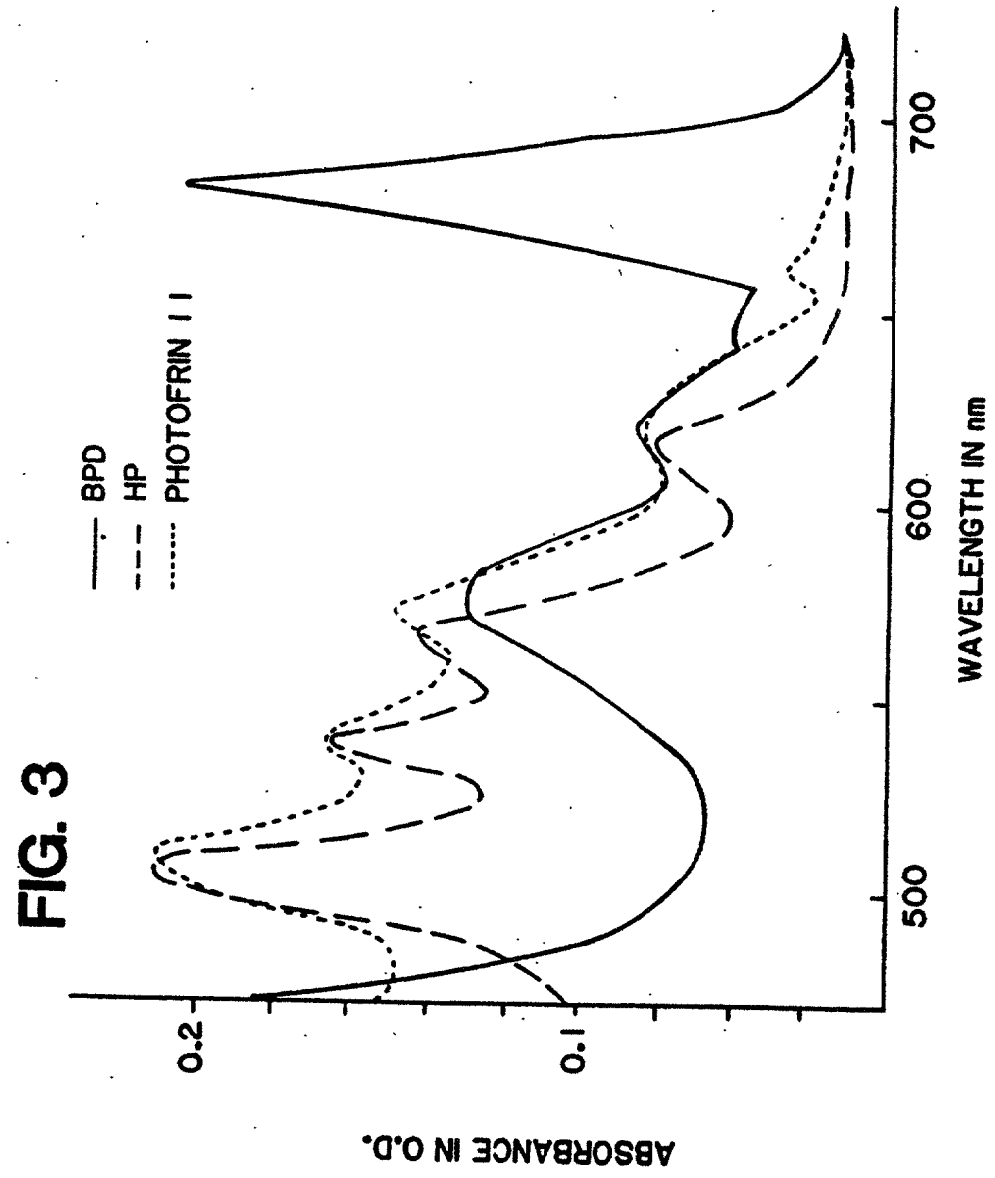
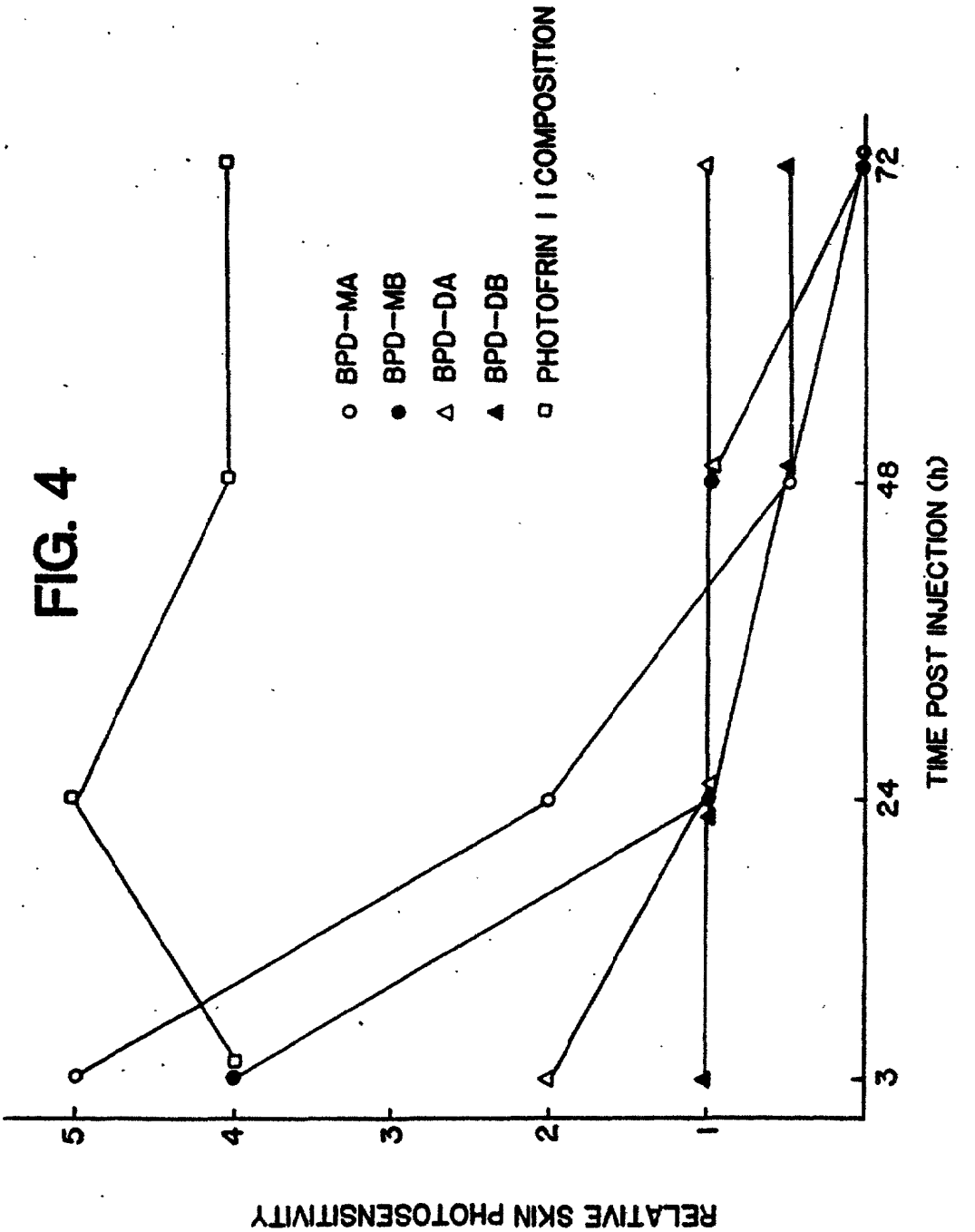

BPD
HP
PHOTOFRIN II
0.2
0.1
500
600
700
WAVELENGTH IN nm
ABSORBANCE IN O.D.

FIG. 3

FIG. 4


BPD-MA
BPD-MB
BPD-DA
BPD-DB
PHOTOFRIN II COMPOSITION
RELATIVE SKIN PHOTOSENSITIVITY
TIME POST INJECTION (h)
1
2
3
4
5
3
24
48
72

WAVELENGTH-SPECIFIC CYTOTOXIC AGENTS

CROSS-REFERENCE TO RELATED PATENT APPLICATION

This is a continuation-in-part U.S. Ser. No. 221,161, filed July 19, 1988,now U.S. Pat. No. 4,920,143, which is a continuation-in-part of U.S. Ser. No. 041,680, filed Apr. 23, 1987 no U.S. Pat. No. 4,883,790, which is a continuation-in-part of U.S. Ser. No. 005,204, filed Jan 20, 1987, now abandoned.

FIELD OF THE INVENTION

The invention relates to the use of light absorbing compounds to mediate the destruction of unwanted cells or tissues or, other undesirable materials by irradiation. Specifically, the invention relates to the use of hydro-monobenzoporphyrin derivatives having absorption maxima in the range 670–780 nanometers to mediate the irradiation of materials to be destroyed, and to the use of these compounds conjugated to target-specific ligands, such receptor-specific ligands, or immunoglobulins or their immunospecific fragments, to focus the effects of the irradiation on particular targets.

BACKGROUND OF THE INVENTION

The use of hematoporphyrin and its acetylated derivative mixture hematoporphyrin derivative (HPD) systemically, combined with irradiation, for the detection and treatment of malignant cells has, by this time, some considerable history. HPD is a mixture of porphyrins including hematoporphyrin itself, hyiroxyethyl vinyl deuteroporphyrin, protoporphyrin, and dihematoporphyrin ethers. (See, e.g., "Porphyrin Photosensitization", Kessel, D., et al, eds. (1983) Plenum Press.)

HPD seems "naturally" capable of localizing in malignant cells. When irradiated, it has two properties which make it useful. First, when irradiated with ultraviolet or visible light, it is capable of fluorescence, and thus is useful in diagnostic methods related to detection of malignancy (see, for example, Kessel, et al (*supra*); Gregory, H.B. Jr., et al, *Ann Surq*(1968) 167:827–829). More pertinent to the present invention is the capacity of HPD, when irradiated with visible light, to exhibit a cytotoxic effect on the cells in which it is localized (see, for example, Diamond, I., et al, *Lancet*(1972) 2:1175–1177; Dougherty, T.J., et al, *Cancer Research*(1978) 38:2628–2635; Dougherty, T.J., et al, "The Science of Photo Medicine": (1982) J.D. Regan & J.A. Parrish, eds., pp. 625–638; Dougherty, T.J., et al, "Cancer: Priniciples and Practice of Oncology" (1982) V.T. DeVita Jr., et al, eds., pp. 1836–1844). Although it has not been definitively established, the effect of HPD in killing cells seems to be due to the formation of singlet oxygen upon irradiation (Weishaupt, K.R., et al, *Cancer Research*(1976) 36:2326–2329). Several mechanisms for this effect have been proposed, and it has recently been shown that the active ingredient in HPD which mediates the cytotoxic effect of visible light irradiation is the mixture of dihematoporphyrin ethers (DHE) (Dougherty, T.J., et al, "Porphyrin Localization and Treatment of Tumors" (1984) pp. 301–314; Dougherty, T.J. *CRC Critical Reviews in Oncolocy/Hematolocy*(1984) 2:83–116).

A purified form of the active component(s) of HPD is obtained by adjustment of pH to cause aggregation and recovery of the aggregate, as disclosed in U.S. Pat. 4,649,151. The purified form called DHE in the patent, is marketed under the trademark Photofrin ® II and has been used in a manner completely analogous to HPD.

In addition to in vivo therapeutic and diagnostic protocols for tumors as described in the above-cited patent, the porphyrins, including HPD and its more purified derivatives, can be used in other in vivo and in vitro applications. For example, photosensitizers are useful in the detection and treatment of atherosclerotic plaques as described in U.S. Pat. Nos. 4,512,762 and 4,577,636. U.S. Pat. Nos. 4,500,507 and 4,485,806 describe the use of radiolabeled porphyrin compounds, including HPD, for tumor imaging. U.S. Pat. No. 4,753,958 to the University of California describes the use of topical application of porphyrin sensitizers for diagnosis and treatment of skin diseases. U.S. Pat. No. 4,748,120 describes the use of photosensitizers in the treatment of whole blood or blood components. Photochemical decontamination treatment of blood and components is also described in U.S. Pat. No. 4,727,027 where the photosensitizer is furocumarin and its derivatives. In addition, viruses are inactivated in therapeutic protein compositions in vitro as disclosed in U.S. Pat. No. 4,268,947.

While the treatment of tumors and other undesirable targets with HPD relies on the intrinsic ability of HPD to localize in malignant cells, a considerable improvement and refinement in specificity has been achieved by conjugating the hematoporphyrin to tumor-specific antibodies. For example, when hematoporphyrin was coupled to monoclonal antibodies directed to a murine myosarcoma cell line Ml, administration of anti-Ml hematoporphyrin-conjugates to tumor-bearing animals followed by exposure to incandescent light resulted in the suppression of Ml growth (Mew, D., et al, *J Immunol*(1983) 130:1473–1477). In additional work, hematoporphyrin was conjugated to a monoclonal antibody specific to an antigen associated with a human leukemia (CAMAL) and the conjugates were shown to mediate the irradiation-induced killing of leukemic cells specifically, in vitro (Mew, D., et al, *Cancer Research* (1985) 45:4380–4386). Conjugation of the related compound chlorine 6 to anti-T cell Mab has also been reported (Oseroff, A.R., et al.,*Proc Natl Acad Sci USA* (1986) 8]:8744–8748).

While the conjugation of hematoporphyrin to immunoglobulins specific for targeted cells refines the ability of the hematoporphyrin to home to the desired cells or tissue, this still does not solve another problem ancillary to this general therapeutic approach, namely that the wavelength for irradiation required to activate the hematoporphyrin or HPD, which is in the range of 630 nanometers, is also an energy which is readily absorbed by the porphyrins and other natural chromophores in the blood and other tissues. Therefore, relatively large amounts of the hematoporphyrin or HPD must be administered, often resulting in oversensitization of the patient to light in general. It would be desirable to administer compounds to mediate the effects of irradiation in a lower amount, thus avoiding the problems of hypersensitivity exhibited nonspecifically throughout the subject organism. The activity of certain of these compounds was described in a paper by Richter, A.M., et al, in *J Natl Cancer Inst* (1987) 79:1327–1332, mailed to subscribers on Jan. 19, 1988. The invention is directed to the use of such compounds.

DISCLOSURE OF THE INVENTION

The invention provides light absorbing compounds capable of exhibiting light-mediated cytotoxic and diagnostic effects. In addition to their in vitro use, these compounds may be administered in in vivo relatively low dosage due to their capability to absorb radiation whose energy range is outside of that normally absorbed by the components present in high concentration in the blood or other tissues, in particular, the porphyrin residues normally associated with hemoglobin and myoglobin. Therefore, by providing these modified porphyrins for in vivo treatment at lower concentration, hypersensitivity of nontarget tissues is reduced, and the irradiation treatment can be conducted at a wavelength at which the native chromophores do not compete for photons with the active compounds, resulting in greater depth of penetration of the light. Similar advantages accrue in in vitro treatment of colored materials, such as blood samples.

These photoactive compounds are modified porphyrins which, by virtue of their derivatization, undergo a shift in absorption maxima so that they appear green rather than red, indicating their absorption of wavelengths in the red-orange range. This collection of derivatives has therefore been nicknamed "green porphyrin" (Gp) and has been shown to confer sensitivity on target cells at concentrations greater than 10-fold lower than those required for hematoporphyrin (Hp) or HPD.

The Gp is selected from a group of porphyrin derivatives obtained using Diels-Alder reactions of acetylene derivatives with protoporphyrin under conditions which effect a reaction at only one of the two available conjugated, nonaromatic diene structures present in the protoporphyrin-IX ring system (rings A and B). The formulas shown in FIG. 1 represent the green porphyrins of the invention. Also, for convenience, an abbreviation of the term hydro-monobenzoporphyrin derivative—"BPD"—is generally used to refer to compounds of formulas 3 and 4 of FIG. 1, as these are the preferred forms of Gp.

Furthermore, dimeric forms of the Gp can be provided, thus amplifying the ability of the Gp compound to absorb light on a per mole basis. Dimeric and multimeric forms of Gp/porphyrin combinations can also be employed, providing additional absorption wavelengths.

In addition, the modified porphryins (referred to as "green porphyrin" or "Gp" herein) of the invention can be conjugated to specific ligands reactive with a target, such as receptor-specific ligands or immunoglobulins or immunospecific portions of immunoglobulins, permitting them to be more concentrated in a desired target tissue or substances. This conjugation permits further lowering of the required dose levels since the material is not wasted in distribution into other tissues whose destruction, far from being desired, must be avoided.

Thus, in one aspect, the invention relates to methods of locating or effecting cytotoxicity, i.e. photosensitizing, with respect to target materials using the hydro-monobenzoporphyrins of the invention either alone or as conjugates. The hydro-monobenzoporphyrins are green porphyrins (Gp) as shown in FIG. 1, and are localized specifically in vivo to certain target tissues, where their presence can be detected by fluorescence, or by other means when the Gp is provided with additional or alternate labeling. As indicated above, the specificity of the Gp can be further enhanced by conjugation to ligands specific for the target. In addition, when the Gp is irradiated in situ using light in the range of 670–780 nm, photoactivation results in cytotoxicity to the surrounding tissue. Cells to which the Gp is normally attracted include tumor cells, and neoplastic cells in general, as well as bacteria and other diseased tissues. The method can be applied either in vivo or in vivo, and, when applied in vivo, can be topical or systemic.

In another aspect, the invention relates to certain specific Gp compounds including those of formulas 3 and 4 designated herein "BPD", that are partially hydrolyzed forms containing free (non-esterified) carboxylic acid moieties or their salts in the R^3 substituents. The invention also relates to labeled forms of these compounds.

In other aspects, the invention relates to conjugates of the formulas Re*-L-Gp and Ig-L-Gp wherein Re* represents a ligand which is specific to, and capable of, binding a receptor at a cell surface, Ig represents an immunoglobulin or an immunologically reactive portion thereof, Gp represents a hydro-monobenzoporphyrin having an absorption maximum in the range of 670–780 nanometers, and L represents either a covalent bond linking these components or a linking moiety covalently linked to each of the Re* or Ig and Gp.

The invention is also directed to tripartite complexes which include Re*-L-Gp or Ig-L-Gp further conjugated to or associated with a label. The label may be bound either to the targeting component or to the Gp or both.

In another aspect, the invention relates to pharmaceutical compositions containing these active ingredients.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1–2 to 1–6 show the structure of green porphyrin (Gp) compounds used in the methods and conjugates of the invention.

FIGS. 2–2 to 2–4 show the structure of four preferred forms of the hydro-monobenzoporphyrin derivative of formulas 3 and 4 (BPD).

FIG. 3 shows a comparative absorption spectrum of a BPD compound and prior art compositions.

FIG. 4 shows the results of skin sensitivity assay using a BPD compound.

MODES OF CARRYING OUT THE INVENTION

The Hydro-monobenzoporphyrins (Gp)

All of the compositions of the invention employ as the light absorbing compound, a derivative of the protoporphyrin ring system which has a light absorption maximum in the range of 670–780 nanometers. FIG. 3 shows the absorption spectrum of one of the compounds of the invention shown in FIG. 2, BPD-DA, wherein R^1 and R^2 are carbomethoxy, in comparison to HPD and Photofrin ® II compositions. Only BPD-DA has a major absorption peak at about 685 nm.

In general, this shift is achieved by effectively saturating one of the two π-bonds in one, but not two, of the four pyrrole rings which constitute the typical porphyrin system. In protoporphyrin-IX two of the pyrroles contain vinyl substitutions such that the exocyclic π-bond is conjugated to one of the two π-bonds in the ring. A Diels-Alder reaction involving one of these conjugated systems with an acetylene derivative dienophile results in a fused cyclohexadiene—referred to herein as "hydrobenzo"—fused to the A or B ring, as shown in formulas 1 and 2. Rearrangement of the π

system in the hexadiene ring results in the compounds of FIGS. 3 and 4; reduction provides the compounds of formulas 5 and 6. All of these compounds provide the desired shift in absorption maximum.

Specific preparation of some compounds useful in the invention or their precursors is described by Morgan, A.R., et al, *J Chem Soc Chem Commun* (1984) pp. 1047–1048; and by Pangka, B.S. et al, *J Organic Chem* (1986) 51:1094. As described in these publications, it had earlier been reported that protoporphyrin-IX dimethyl ester, when reacted with strong Diels-Alder dienophile reagents such as tetracyanoethylene, is derivatized to the hydro-dibenzo derivatives. However, it is clear that, as shown by these references, when acetylene is derivatized with more weakly electron withdrawing groups and used as a Diels-Alder reagent, hydro-monobenzo derivatives are formed. Thus, there are obtained directly from reaction of protoporphyrin with, for example dimethyl acetylene dicarboxylate (DMAD), compounds shown as formulas 1 and 2 of FIG. 1, wherein R^1 and R^2 represent the substituents on the original acetylene-derived Diels-Alder reagent, $R^1C{=}CR^2$—in this case, carbomethoxy. R^1 and R^2 are, generally, specifically carbalkoxy groups such as carbomethoxy or carboethoxy. R^3 represents substituents present on the porphyrin used in the reaction or substituents derived therefrom. In the Morgan reference, the reaction substrate was protoporphyrin-IX dimethyl ester; thus the ligand R^3 was, in all cases, 2-carbomethoxyethyl.

The disclosed substituents in the Morgan and Pangka references for the acetylene-derived dienophile include phenylsulfonyl—i.e., SO_2Ph, either as a single substituent, as described in the foregoing references (β-phenylsulfonylpropiate) or, putatively, wherein both R^1 and R^2 are sulfonyl derivatives. In general, R^1 and R^2 are each, independently, moderate electron-withdrawing substituents, and are, most commonly, carbalkoxy, or alkyl or aryl sulfonyl, or any other activating substituents, which are not sufficiently electron-withdrawing to result in reaction with both A and B rings rather than reaction with only one, such as cyano or —CONR^{5}CO—wherein R^5 is aryl or alkyl. One of R^1 and R^2 may optionally be H while the other is an electron withdrawing substituent of sufficient strength to facilitate the Diels-Alder reaction.

As used herein, carboxy is, as conventionally defined, —COOH and carbalkoxy is —COOR, wherein R is alkyl; carboxyalkyl refers to the substituent —R'—COOH wherein R6 40 is alkylene; carbalkoxyalkyl refers to —R'—COOR wherein R' and R are alkylene and alkyl respectively. Alkyl is a saturated straight or branched chain hydrocarbyl of 1–6 carbon atoms such as methyl, n-hexyl, 2-methylpentyl, t-butyl, n-propyl, and so forth. Alkylene is as alkyl except that the group is divalent. Aryl or alkyl sulfonyl moieties have the formula SO_2R wherein R is alkyl as above-defined, or is aryl, wherein aryl is phenyl optionally substituted with 1–3 substituents independently selected from halo (fluoro, chloro, bromo or iodo), lower alkyl (1–4C) or lower alkoxy (1–4C). In addition, one or both R^1 of R^2 can itself be aryl —i.e., phenyl optionally subsituted as above-defined.

As shown in FIG. 1, the adduct formed by the reaction of R^1—C≡C —R^2 with the protoporphyrin-IX ring system (R^3 is a protected form of 2-carboxyethyl such as 2-carbomethoxyethyl or 2-carboethoxyethyl; R^4 is $CH{=}CH_2$) are compounds of the formulas 1 and 2 wherein the compound in formula 1 results from addition to the A ring and formula 2 results from addition to the B ring. In these resulting products of formulas 1 and 2, R^4 remains $CH{=}CH_2$, however this vinyl group is readily 7 derivatized to other embodiments of R^4 by addition to or oxidation of the vinyl ring substituent of ring B in formula 1 or ring A in formula 2. The addition or oxidation products can be further substituted if the added substituents are functional leaving groups—for example —Br may be substituted by —OH, —OR (R is alkyl 1–6C as above), or —NH_2, —NHR, —NR_2, etc. In preferred embodiments, one of the added substituents is hydrogen, and the other is selected from the group consisting of halo (fluoro, chloro, bromo or iodo), hydroxy, lower alkoxy, amino or an amide, sulfhydryl or an organo-sulfide or can be, itself, hydrogen. Addition to the vinyl group does not appreciably change the absorption spectrum of the resulting compound. The product of the Markovnikov addition of water provides a substituent structure analogous to the hematoporphyrin ring system at the relevant ring. Thus, the compounds of the invention include various groups as R^4, including substituents which provide additional prophyrin or prophyrin-related ring systems, as will be further described below.

R^3 in protoporphyrin-IX is 2-carboxyethyl (—CH_2CH_2COOH). However, the nature of R^3 (unless it contains a π-bond conjugated to ring π-bond), is ordinarily not relevant to the progress of the Diels-Alder reaction or to the effectiveness and absorption spectrum of the resulting product. R^3 can thus be, for example, lower alkyl (1–4C), or ω-carboxyalkyl (2–6C) or the esters or amides thereof. The R^3 substituent may also be substituted with halogen as above-defined, or with other nonreactive substituents. However, as the convenient starting materials for the Gp compounds of the invention are the naturally occurring porphyrins, the preferred substituents for R^3 are CH_2CH_2COOH or —CH_2CHR^2COOR, whereing R is alkyl (1–6C).

It should be noted that while the nature of the R^3 substituent does not ordinarily influence the course of the Diels-Alder reaction by altering the nature of the diene substrate, derivatization may be necessary to promote the reaction by providing suitable solubility characteristics or to prevent interference with the reaction. Thus, the Diels-Alder reactions described by Morgan et al and by Pangka et al utilized the dimethylester of protoporphyrin-IX as a substrate in order to prevent interference with the reaction by the free carboxyl group and to provide suitable solubility characteristics.

In the BPD compounds of the invention, it has been found advantageous to hydrolyze or partially hydrolyze the esterified carboxy group in —CH_2CH_2COOR. The hydrolysis occurs at a much faster rate than that of the ester groups of R^1, R^2, and the solubility characteristics of the resulting compounds are more desirable than those of the unhydrolyzed form. Hydrolysis results in the diacid or monoacid products (or their salts).

The hydro-monobenzoporphyrins which directly result from the Diels-Alder reaction described in the cited references can also be isomerized as therein described (see Morgan et al and Pangka et al (sucra)) to compounds of formulas shown as 3 and 4 of FIG. 1 by treatment with suitable reagents such as triethylamine (TEA) in methylene chloride or 1,5-diaza bicyclo [5.4.0]undec-5-ene (DBU). The stereochemistry of the product is determined by the choice of reagent.

The depictions of compounds 3 and 4 in FIG. 1 do not show the relative position of the exocyclic methyl

group (ring A of formula 3 and ring B of formula 4) with respect to the R^2 substituent. It has been found by these authors that rearrangement using TEA gives cis geometry for the angular methyl group and R^2, while treatment with DBU results in the trans product. This cis product is evidently kinetically controlled since treatment of the cis product with DBU results in a further rearrangment to trans stereochemistry. Thus, formulas 3 and 4 of FIG. 1 show the rearranged products generically, from either TEA or DBU catalyzed rearrangement in rings A and B respectively.

In addition, the Diels-Alder products can be selectively reduced by treating with hydrogen in the presence of palladium on charcoal to give the saturated ring analogs, shown as formulas 5 and 6 in FIG. 1, corresponding to the respective Diels-Alder products of rings A and B. These reduced products are less preferred embodiments, and are less useful in the method of the invention than the compounds of formulas 1-4.

The description set forth above with respect to the compounds of formulas 1 and 2 concerning derivatization by conversion of the remaining vinyl substituent (R^4) and with respect to variability of -R^3 applies as well to the compounds of formulas 3, 4, 5 and 6.

The compounds of formulas 3 and 4 (BPD), and especially those which have hydrolyzed and partially hydrolyzed carbalkoxy groups in R^3, are most preferred. Compounds of the invention which contain —COOH may be prepared as the free acid or in the form of salts with organic or inorganic bases.

It will be noted that many of the compounds of FIG. 1 contain at least one chiral center and therefore exist as optical isomers. The conjugates and methods of the invention include compounds having both configurations of the chiral carbons, whether the compounds are supplied as isolates of a single stereoisomer or are mixtures of enantiomers and/or diastereomers. Separation of mixtures of diastereomers may be effected by any conventional means; mixtures of enantiomers may be separated by usual techniques of reacting them with optically active preparations and separating the resulting diastereomers.

It should further be noted that the reaction products may be unseparated mixtures of A and B ring additions, e.g., mixtures of formulas 1 and 2 or 3 and 4 or 5 and 6. Either the separated forms—i.e., formula 3 alone or 4 alone, or mixtures in any ratio may be employed in the methods of therapy and diagnosis set forth herein.

The name "dihydro"-monobenzoporphyrin describes the direct and rearrangement products of the Diels-Alder reaction of the porphyrin ring system with $R^1C{=}C{-}R^2$; "tetrahydro"-monobenzoporphyrin describes the foregoing reduced products of formulas 5 and 6, and "hexahydro"-monobenzoporphyrin describes the analogs containing the exocyclic "benzo" ring completely reduced. Hydro-monobenzoporphyrin is used generically to include all three classes of oxidation state. The monobenzoporphyrins per se are outside the scope of the invention as their absorption maxima do not fall within the required range.

FIG. 2 shows four particularly preferred compounds of the invention which have not been previously described in the art. These compounds are collectively designated benzoporphyrin derivative (BPD) as they are forms of Gp having the formula 3 or 4. These are hydrolyzed or partially hydrolyzed forms of the rearranged products of formula 3 and 4, wherein one or both of the protected carboxyl groups of R^3 are hydrolyzed. The ester groups at R^1 and R^2 hydrolyze relatively so slowly that conversion to the forms shown in FIG. 2 is easily effected.

For purposes of this description, R^3 is $-CH_2CH_2COOR^{3'}$. As shown in FIG. 2, each $R^{3'}$ is H in preferred compound BPD-DA, R^1 and R^2 are carbalkoxy, and derivatization is at ring A; BPD-DB is the corresponding compound wherein derivatization is at ring B. BPD-MA represents the partially hydrolyzed form of BPD-DA, and BPD-MB, the partially hydrolyzed form of BPD-DB. Thus, in these latter compounds, R^1 and R^2 are carbalkoxy, one R^3+is H and the other $R^{3'}$is alkyl (1-6C). The compounds of formulas BPD-MA and BPD-MB may be homogeneous wherein only the C ring carbalkoxyethyl or only the D ring carbalkoxyethyl is hydrolyzed, or may be mixtures of the C and D ring substituent hydrolyzates. In addition, mixtures of any two or more of BPD-MA, -MB, -DA and -DB may be employed in the method of the invention.

As these hydrolyzed forms of the Diels-Alder product are previously undisclosed, the invention is also directed to these compounds. Thus, in another aspect, the invention is directed to compounds of the formulas shown in FIG. 2 wherein R^1 and R^2 are as above defined, and R is alkyl (1-6C). Preferred are embodiments wherein R^1 and R^2 are carbalkoxy, especially carbomethoxy or carboethoxy.

Certain other embodiments wherein R^4 is other than vinyl or wherein R^3 is a non-native substituent are also not disclosed in the art and the invention is directed to them, i.e., the invention is directed to the compounds shown in FIG. 1 wherein

each R^1 and R^2 is independently selected from the group consisting of carbalkoxy (2-6C), alkyl (1-6C) sulfonyl, aryl (6-10C) sulfonyl, aryl (6-10C); cyano; and —$CONR^5CO$— wherein R^5 is aryl (6-10C) or alkyl (1-6C);

each R^3 is independently carboxyalkyl (2-6C) or a salt, amide, ester or acylhydrazone thereof, or is alkyl (1-6C); and

R^4 is $CHCH_2$, $CHOR^{4'}$, —CHO, —$COOR^{4'}$, $CH(OR^{4'})CH_3$, $CH(OR^{4'})CH_2OR^{4'}$, $-CH(SR^{4'})CH_3$, $-CH(NR^{4'}{}_2)CH_3$, $-CH(CN)CH_3$, $-CH(COOR^{4'})CH_3$, $-CH((OOCR^{4'})CH_3$, $-CH(halo)CH_3$, or $-CH(halo)CH_2(halo)$,

wherein $R^{4'}$is H, alkyl (1-6C) optionally substituted with a hydrophilic substituent, or

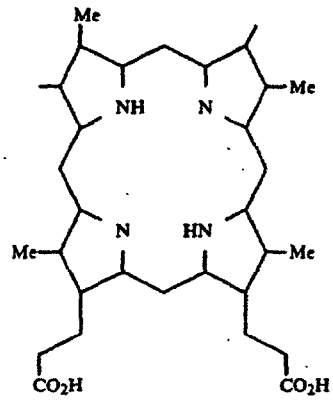
wherein R^4 is an organic group of <12C resulting from direct or indirect derivatization of vinyl, or

wherein R^4 is a group containing 1-3 tetrapyrrole-type nuclei of the formula -L-P as herein defined;

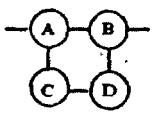
wherein when R^4 is $CHCH_2$, both R^3 cannot be 2-carbalkoxyethyl.

Compounds of the formulas 3 and 4 and mixtures thereof are particularly preferred. Also preferred are those wherein R^1 and R^2 are the same and are carbalkoxy, especially carboethoxy; also preferred are those wherein R^4 is $-CHCH_2$, $CH(OH)CH_3$ or $-CH(halo)CH_3$, or is a group containing 1-3 tetrapyrrole-type nuclei of the formula -L-P (defined below).

As used herein, "tetrapyrrole-type nucleus" represents a four-ring system of the skeleton:



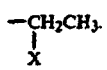
which is abbreviated



and a salt, ester, amide or acylhydrazone thereof, which is highly conjugated. It includes the porphyrin system, which is, in effect, a completely conjugated system, the chlorin system, which is, in effect, a dihydro form of the porphyrin, and the reduced chlorin system, which is a tetrahydro form of the completely conjugated system. When "porphyrin" is specified, the completely conjugated system is indicated; Gp is effectively a dihydro form of the porphyrin system.

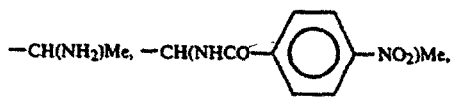
One group of compounds of the invention is that wherein the substituent R^4 includes at least one additional tetrapyrrole-type nucleus. The resulting compounds of the invention are dimers or oligomers in which at least one of the tetrapyrrole-type ring systems is Gp. Linkage between the Gp moiety through the position of R^4 to an additional tetrapyrrole-type ring system may be through an ether, amine or vinyl linkage. Additional derivatization in the case of porphyrin ring systems which have two available substituent positions (in both A and B rings) corresponding to R^4 can also be formed, as further described below.

As stated above, the compounds of formulas shown in FIG. 1 include those wherein the embodiment of R^4 is formed by addition to the vinyl groups of initial Gp products. Thus, R^4 can be any substitutent consistent with that formed by a facile addition reaction. Thus, both added substituents can be, for example, OH or halo, and these substituents can be further substituted, or the addition reagent may be of the form HX wherein H is added to the ring-adjacent carbon to provide R^4 of the form

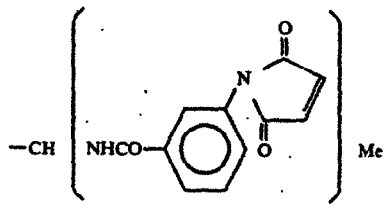


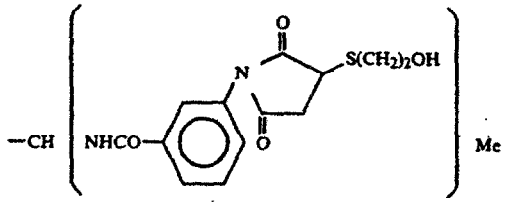
The vinyl group can also be oxidized to obtain R^4 as CH_2OH, —CHO, or COOH and its salts and esters.

Thus, in general R^4 represents any substituents to which the vinyl group —CH=CH_2 is readily converted by cleavage or addition, and further resultants of reaction of leaving groups with additional moieties. Typical R^4 substituents include:



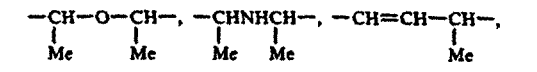
—CH(imidazole)Me,



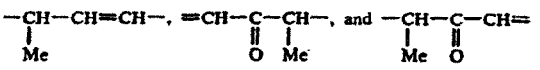


CH(OH)Me, —CHBrMe, —CH(OMe)Me, —CH-(pyridinum bromide)Me, —CH(SH)Me and the disulfide thereof, —$CHOHCH_2OH$, —CHO, and —COOH or —COOMe.

When R^4 is -L-P, the substituent formula "-L-P" represents a substituent wherein -L- is selected the group consisting of

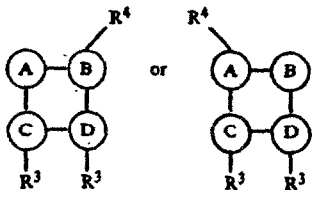


(a) (b) (c)

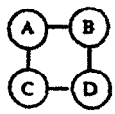


(d) (e) (f)

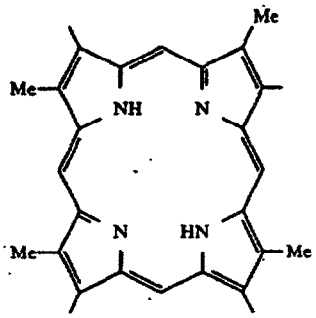
and P is selected from the group consisting of Gp wherein Gp is of the formula 1-6 shown in FIG. 1, but lacking R^4 and conjugated through the position shown in FIG. 1 as occupied by R^4 to L, and a porphyrin of the formula



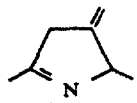
wherein R^3 and R^4 are as above-defined, and the unoccupied bond is then conjugated to L. It is understood that the abbreviation



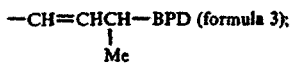
represents a porphyrin of the formula:

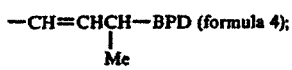


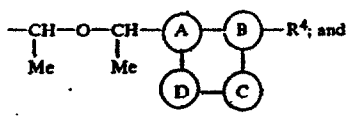
(It is also understood that when -L- is of the formula (e) or (f), the ring system to which the double bond is attached will have a resonance system corresponding to

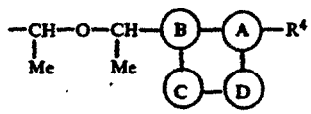


in the ring to which the double bond is attached, as shown.)

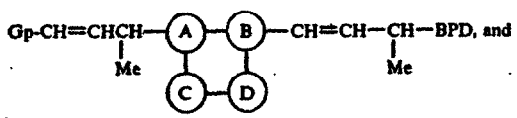




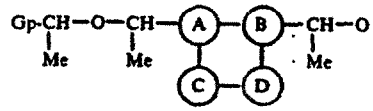


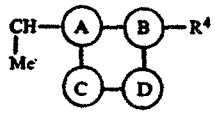


wherein R^4 is as above defined. Thus, compounds of the invention include:



-continued



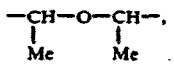


and the like.

Preparation of the Dimers and Oligomers

The dimers and oligomeric compounds of the invention can be prepared using reactions analogous to those for dimerization and oligomerization of porphyrins per se.

For formation of compounds of the invention where -L- is of the formula



i.e., an ether linkage, the Gp vinyl group is converted to the halide, preferably the chloride, by treating the Gp in a solution of, for example, methylene chloride with HBr to recover the addition product. The resulting product is harvested by evaporation in vacuo. redissolved in methylene chloride and added to an insoluble base such as solid potassium carbonate. To this is added an equivalent of the tetrapyrrole-type nucleus "P" to be linked wherein the reactive R^4 moiety of "P" is 1-hydroxyethyl. The mixture is stirred for the appropriate amount of time, around 12 hours, generally, and the resulting diastereomeric pair of dimers (the enantiomeric paired form and a meso form) can be separated from the mixture chromatographically. The tetrapyrrole-type nucleus represented by "P" in this procedure can be either another Gp or a porphyrin.

If the "P" substitutent is a porphyrin, an additional vinyl group may be made available for further halogenation and further reaction to form higher order oligomers.

For embodiments wherein -L- contains a vinyl 15 group, the dimers are obtained by treating Gp wherein R^4 is 1-hydroxyethyl with an equivalent amount of the linking tetrapyrrole-type nucleus also having the linking R^4 as 1-hydroxyethyl with a strong, nonnucleophilic acid, such as trifluoromethyl sulfonic acid. This treatment results in precipitation of the resulting methylpropenyl linked dimer. (The ether-linked dimer can be formed as a side product in this reaction by substituting alternative acids such as sulfuric acid.)

The amino-linked compounds can be formed by treatment of the vinyl group with HBr followed by treatment with the appropriate amine to obtain the desired linkage.

The Tarcet-Specific Component

The target-specific component can be, for example, an immunoglobulin or portion thereof or a ligand specific for receptor.

The immunoglobulin component can be any of a variety of materials. It may be derived from polyclonal or monoclonal antibody preparations and may contain whole antibodies or immunologically reactive fragments of these antibodies such as F(ab')2, Fab, or Fab' fragments. Use of such immunologically reactive fragments as substitutes for whole antibodies is well known in the art. See, for example Spiegelberg, H.L., in "Immunoassays in the Clinical Laboratory" (1978) 3:1-23.

Polyclonal anti-sera are prepared in conventional ways by injecting a suitable mammal with antigen to which antibody is desired, assaying the antibody level in serum against the antigen, and preparing anti-sera when the titers are high. Monoclonal antibody preparations may also be prepared conventionally such as by the method of Koehler and Milstein using peripheral blood lymphocytes or spleen cells from immunized animals and immortalizing these cells either by viral infection, by fusion with myelomas, or by other conventional procedures, and screening for production of the desired antibodies by isolated colonies. Formation of the fragments from either monoclonal or polyclonal preparations is effected by conventional means as described by Spiegelberg, H.L., supra.

Particularly useful antibodies exemplified herein include the monoclonal antibody preparation CAMAL-1 which can be prepared as described by Malcolm, A., et al, *Ex Hematol* (1984) 12:539-547; polyclonal or monoclonal preparations of anti-MI antibody as described by Mew, D., et al, *J Immunol* (1983) 130:1473-1477 (supra) and B16G antibody which is prepared as described by Maier, T., et al, *J Immunol* (1983) 131:1843; Steele, J.K., et al, *Cell* Immunol (1984) 90:303.

The foregoing list is exemplary and certainly not limiting; once the target tissue is known, antibody specific for this tissue may be prepared by conventional means. Therefore the invention is applicable to effecting toxicity against any desired target.

The ligand specific for receptor, Re*, refers to a moiety which binds a receptor at cell surfaces, and thus contains contours and charge patterns which are complementary to those of the receptor. The ligand specific for receptor is symbolized in the formulas of the compounds of the invention as Re*, wherein the asterisk indicates that the moiety bound in the compound of the invention is not the receptor itself, but a substance complementary to it. It is well understood that a wide variety of cell types have specific receptors designed to bind hormones, growth factors, or neurotransmitters. However, while these embodiments of ligands specific for receptor are know and understood, the phrase "ligand specific for receptor", as used herein, refers to any substance, natural or synthetic, which binds specifically to a receptor.

Examples of such ligands include the steroid hormones, such as progesterone, estrogens, androgens, and the adrenal cortical hormones; growth factors, such as epidermal growth factor, nerve growth factor, fibroblast growth factor, and so forth; other protein hormones, such as human growth hormone, parathyroid hormone, and so forth; and neurotransmitters, such as acetylcholine, serotonin, and dopamine. Any analog of these substance which succeeds in binding to the receptor is also included.

Linkage

The conjugation of the target-cell-specific component to the hydro-monobenzoporphyrin can be effected by any convenient means. For proteins, such as Ig and certain Re*, a direct covalent bond between these moieties may be effected, for example, using a dehydrating agent such as a carbodiimide, in which case L represents a covalent bond. A particularly preferred method of covalently binding hydro-monobenzoporphyrins ,) to the immunoglobulin moiety is treatment with 1-ethyl-3-(3-dimethylamino propyl) carbodiimide (EDCI) in the presence of a reaction medium consisting essentially of dimethyl sulfoxide (DMSO). A preparation using this preferred procedure is illustrated in Example 3 below.

Of course, other dehydrating agents such as dicyclohexylcarbodiimide or diethylcarbodiimide could also be used as well as conventional aqueous and partially aqueous media.

Nonprotein receptor ligands can be conjugated to the Gp according to their relevant functional groups by means known in the art.

The active moieties of the conjugate may also be conjugated through linker compounds which are bifunctional, and are capable of covalently binding each of the two active components. A large variety of these linkers is commercially available, and a typical list would include those found, for example, in the catalog of the Pierce Chemical Co. These linkers are either homo or heterbifunctional moieties and include functionalities capable of forming disulfides, amides, hydrazones, and a wide variety of other linkages.

Other linkers include polymers such as polyamines, polyethers, polyamine alcohols, derivatized to the components by means of ketones, acids, aldehydes, isocyanates, or a variety of other groups.

The techniques employed in conjugating the active moieties of the conjugate include any standard means and the method for conjugation does not form part of the invention. Therefore, any effective technique known in the art to produce such conjugates falls within the scope of the invention, and the linker moiety is accordingly broadly defined only as being either a covalent bond or any linker moiety available in the art or derivable therefrom using standard techniques.

Label

For use in the method of the invention either the green porphyrin compounds per se or the conjugates may be further derivatized to a compound or ion which labels the drug. A wide variety of labeling moieties can be used, including radiosotopes, chromophores, and fluorescent labels. Radioisotope labeling is preferred, as it can be readily detected in vivo.

The compounds which are Gp alone or are conjugates of Gp with a specific binding substance can be labeled with radioiostopes by coordination of a suitable radioactive cation in the porphyrin system. Useful cations include technetium, gallium, and indium. In the conjugates, either or both the specific binding substances can be linked to or associated with label, or the label can be conjugated or coordinated with the Gp moiety itself.

Metal Ions

The compounds of the invention can be administered or used in in vitro methods as shown above or when complexed to appropriate metal ions. As is generally understood in the art, the tetrapyrrole-type nucleus can be treated with an appropriate ion such as magnesium ion, zinc ion, stannous ion, and the like to obtain the

metal complex. As stated above, the metal ion may also be a radiolabel. The nature and desirability of the inclusion of a metal ion in the tetrapyrrole-type nucleus depends on the specific application for which the compound is intended. When the inclusion of a metal ion is desired, the desired metal ion can be inserted using the appropriate metal salts under known conditions. For example, zinc ion can be introduced by treating the compound with zinc acetate in 1:1 methylene chloride:methanol.

Administration and Use

The improved photosensitizing compounds of the invention are thus useful in general, in the manner known in the art for hematoporphyrin derivative and for DHE. These materials are useful in sensitizing neoplastic cells or other abnormal tissue to destruction by irradiation using visible light—upon photoactivation, the compounds have no direct effect, nor are they entered into any biological event; however the energy of photoactivation is believed to be transferred to endogenous oxygen to convert it to singlet oxygen. This singlet oxygen is thought to be responsible for the cytotoxic effect. In addition, the photoactivated forms of porphyrin fluorescence which fluoresce can aid in localizing the tumor.

Typical indications, known in the art, include destruction of tumor tissue in solid tumors, dissolution of plaques in blood vessels (see, e.g., U.S. Pat. No. 4,512,762); treatment of topical conditions such as acne, atheletes foot, warts, papilloma, and psoriasis and treatment of biological products (such as blood for transfusion) for infectious agents, since the presence of a membrane in such agents promotes the accumulation of the drug.

The conjugate of the invention, of the hydromonobenzoporphyrins when employed alone are formulated into pharmaceutical compositions for administration to the subject or applied to an in vitro target using techniques known in the art generally. A summary of such pharmaceutical compositions may be found, for example, in *Remington's Pharmaceutical Sciences.* Mack Publishing Co., Easton, PA, latest edition.

The conjugates or compounds of the invention taken alone can be used in the systemic treatment of tumors and neoplastics made as bronchial, cervical, esophageal or colon cancer and for the diagnosis of same.

The conjugates and hydro-monobenzoporphyrins of the present invention, labeled or unlabeled, can be administered systemically, in particular by injection, or can be used topically. The Gp or conjugates can be used singly or as components of mixtures.

Injection may be intravenous, subcutaneous, intramuscular, or even intraperitoneal. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid form suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol and the like. Of course, these compositions may also contain minor amounts of nontoxic, auxiliary substances such as wetting or emulsifying agents, pH buffering agents and so forth.

Systemic administration can also be implemented through implantation of a slow release or sustained release system, by suppository, or, if properly formulated, orally. Formulations for these modes of administration are well known in the art, and a summary of such methods may be found, for example, in *Remington's Pharmaceutical Sciences (supra).*

For diagnosis, the compounds may be used along or may be labeled with a radiosotope or other detecting means.

If treatment is to be localized, such as for the treatment of superficial tumors or skin disorders, the active conjugates or hydro-monobenzoporphyrins may be topically administered using standard topical compositions involving lotions, suspension, or pastes.

The quantity of conjugates or green porphyrin derivative to be administered depends on the choice of active ingredient, the condition to be treated, the mode of administration, the individual subject, and the judgment of the practitioner. Depending on the specificity of the preparation, smaller or larger doses may be needed. For compositions which are highly specific to target tissues, such as those which comprise conjugates of the green porphyrin with a highly specific monoclonal immunoglobulin preparation or specific receptor ligand, dosages in the range of 0.05–1 mg/kg are suggested. For compositions which are less specific to the target tissue, larger doses, up to 1–10 mg/kg may be needed. The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large and considerable excursions from these recommended values are expected.

In addition to in vivo use, the compounds of the invention can be used in the treatment of materials vitro to destroy harmful viruses or infectious agents. For example, blood plasma or blood which is to be used for transfusion or banked for future transfusion can be treated with the compounds of the invention and irradiated to effect sterilization. In addition, biological products such as Factor VIII which are prepared from biological fluids can be irradiated in the presence of the compounds of the invention to destroy contaminants.

EXAMPLES

The following example are intended to illustrate the invention but not to limit its scope.

EXAMPLE 1

In Vitro Photosensitization by Green Porphyrins

Target cells were washed three times in serum-free medium (DME), counted and made up to a concentration of 10^7 cells per ml.

For the "affinity" assay, in the dark, 100 μl of the target cell suspension and 100 μl of the test or control compound were mixed. "Labeling" was allowed to continue for one hour at 4° C, and labeled cells were washed in the dark three times with 3 ml medium each time and resuspended in fresh medium. The resuspended cells were then subjected to light exposure at 300–750 nanometers for 30 minutes.

In a "direct" assay the target cells were irradiated immediately upon addition of the test or control compound.

The effect of irradiation was estimated using methods appropriate to the target cells.

When human erythrocytes (RBCs) were used as target cells, the hemolysis caused by irradiation of control (hematoporphyrin, Hp) labeled and green porphyrin (Gp) labeled cells were estimated visually. The Gp used in this Example was the BPD-DB of FIG. 2 wherein R^1 and R^2 are carboethoxy. Repeated tests showed this green porphyrin to be 20–30 times more active than Hp

in this assay. Thus, a concentration of 250 ng/ml Hp was required under the above conditions to obtain 50% hemolysis while only 10 ng/ml of green porphyrin was required to hemolyze 50% of the RBCs.

When the murine mastocytoma cell line P815 was used, the results were determined as follows:

The cells were labeled as above using concentration of 10-50 ng/ml of Hp as control and the BPD-DB as the test substance. The resuspended cells were treated with 300–750 nm light for 30 minutes and the viability resulting was estimated by direct counting using eosin-Y exclusion, a standard procedure for differentiating living from dead cells.

In other determinations conducted as above, the cells recovered from light exposure were assayed for L viability by incubating them for 18 hours in 10 μCi/ml tritium-labeled thymidine according to the standard procedure whereby thymidine incorporation is equated with viability. The cells were harvested and radioactivity uptake was measured by a scintillation counter.

Fifty percent of the P815 cells were killed at 580 ng/ml Hp, but at only 32 ng/ml green porphyrin (BPD-DB).

The results of each determination on a variety of cells is shown in Table 1 (LD^{50} in the concentration of compound required to kill 50% of the cell population.)

TABLE 1

	LD_{50} (ng/ml)			
	Direct test		Affinity test	
Cell line	Gp	Hp	Gp	Hp
Normal lymphocytes	4.2	31	11	100
HL-60	3.5	64	7.2	145
K562	70	770	33	2,500
KG-1	163	960	80	2,350
P815	32	580	26	1,300

EXAMPLE 2

Selective Binding of Green Porphyrin

P815 cells were incubated as described in Example 1 using 1–200 ng/ml Hp or Gp. The Gp was BPD-DB of FIG. 2 wherein R^1 and R^2 are carboethoxy. The cells were labeled in the dark for 30 minutes, washed free of unabsorbed porphyrins, resuspended, and then exposed to 300–750 nm light for another 30 minutes. Viability of the cells was established by tritiated thymidine incorporation after labeling with 20 μCi/ml tritiated thymidine and incubating at 37° C. for 18 hours.

The results showed that 50% of the P815 cells were destroyed at 6–20 ng/ml BPD-DB or at 200 ng/ml hematoporphyrin.

EXAMPLE 3

Preparation of Immunoconjugates

This example describes methods of preparation for immunoconjugates of four different anitibody preparations with either hematoporphyrin (Hp) or green prophyrin (Gp); in this example, Gp is BPD-DB of FIG. wherein R^1 and R^2 are carboethoxy. The antibodies employed were CAMAL-1, anti-Ml antibody, and B16G antibody, all prepared as described hereinabove, and affinity purified rabbit/anti-mouse Ig (RαMIg). In addition, a purified irrelevant monclonal preparation (C-MAb) was used where a control was desired.

One preparation of the conjugates is basically as described in Mew, D., et al, *J Immunol* (1983) 130:1473 (supra). Briefly, to 220 mg pH 0.2 HCl (Sigma Chemical Co., St. Louis, MO) in 25 ml water and 0.8 ml N,N-dimethylformamide was added 20 mg 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide HCl (EDCI) in 0.6 ml water. After 30 minutes, this solution was mixed with 15 mg of the antibody protein dissolved in 5 ml distilled water and incubated for 5 hours. During this period, the pH of the solution was monitored and adjusted to between 6 and 7. Then 50 μl of monoethanolamine were added, and the solution was allowed to stand overnight at room temperature. The solution was dialyzed against 0.001 M phosphate buffer pH 7.4 for four days with three changes per day and overnight against PBS. The conjugate of green porphyrin is analogously prepared.

In a preferred method, the conjugation is conducted in an entirely nonaqueous solvent.

In a typical protocol, 2 ml of a dispersion in DMSO containing 5 mg each of the Hp or Gp and the dehydrating agent is prepared and stirred for 30 minutes at room temperature under nitrogen. To this is added a dispersion containing 2 mg of the appropriate immunoglobulin in 2 ml of DMSO, and the resulting mixture stirred for another 10 minutes. This mixture is then worked up by dilution in phosphate-buffered saline, pH 7.4 (PBS) by adding 5 times the volume of PBS containing 50 μl monoethanolamine, and is then dialyzed against PBS using three changes of wash.

Alternatively, 2 ml of a dispersion containing 5 mg each of Hp or Gp, a linking agent, and a dehydrating agent is prepared and stirred for approximately 15 minutes at room temperature under nitrogen. To this is then added a dispersion containing about 2 mg of the immunospecific protein in 2 ml of tetrahydrofuran and the resulting mixture stirred for another 10 minutes. The mixture is then worked up as described above.

The foregoing procedures are appropriate for CAMAL-1 and for the remaining antibody preparations above listed.

In addition, the following preparations were made specifically with B16G and RαMIg:

B16G

11 mg of hematoporphyrin plus 11 mg EDCI in 4 ml spectral grade DMSO was stirred for 30 minutes under nitrogen at room temperature before the addition of 20 mg lyophilized B16G antibodies, prepared as described by Maier, T., et al, *J Immunol* (1983) 131:1843, in 2 ml DMSO. The resulting mixture was stirred for 40 seconds at room temperature and worked up as described above. The resulting product contained 375 μg Hp/mg B16G. A similar procedure is used substituting Gp for Hp.

RαMIg

400 μg of EDCI and 400 μg hematoporphyrin in 1 ml DMSO were stirred for 30 minutes under nitrogen at room temperature as above before the addition of 800 μg lyophilized RαMIg antibodies, prepared as described by Mew, D., et al, *J Immunol* (1983) 1473–1477, in 1 ml DMSO. The resulting mixture was stirred for 30 seconds and worked up as described above to obtain a product containing 200 μg Hp/mg RαMIg. A similar procedure is used substituting Gp for Hp.

EXAMPLE 4

Specificity of Immunoconjugates in Vitro

In the following determinations, the levels of antibody conjugation were as follows, expressed as μg Hp or green porphyrin (Gp) per mg immunoglobulin:
RαMIg-Hp: 110 μg/mg;
B16G-p, 156 μg/mg;
CAMAL-1-Hp, 260 μg/mg;
Anti-Ml-Hp, 170 μg/mg;
C-MAb-Hp, 95 μg/mg;
RαMIg-Gp, 120 μg/mg;
B16G-Gp, 165 μg/mg;
CAMAL-1-Gp, 75 μg/mg;
C-MAb-Gp 90 μg/mg.

The Ig-Hp and Ig-Gp conjugates are tested against cells in vivo by mixing the conjugates with the appropriate cell types, along with suitable controls, and then exposing the labeled cells to irradiation. Procedures for carrying out this assay were described in detail in Mew, D., et al, *Cancer Research* (1985) for CAMAL-1, and by Mew, D., et al, *J Immunol* (1983) for Anti-Ml, both references cited hereinabove and incorporated herein by reference.

Briefly, for CAMAL-1, three cell lines, WC4, WC6 and WC2 (WC4 and WC6 produces the CAMAL antigen, but WC2 does not), are labeled with the appropriate Ig-Hp or Ig-Gp preparation as described above in Example 1. The labeled cell preparations containing 10^6 cells each are introduced to Rose chambers and exposed to light activation with a laser at 630 nm. The results for various preparations are then compiled.

For the anti-Ml conjugate, Ml tumor cells are used as target cells and treated with the Ig-Hp, Ig-Gp conjugates or drug or antibody alone or the combination of antibody and drug, but uncoupled, by incubating them in 6% CO_2 humidified incubator at 37° for two hours. The cells are washed three times in PBS and then plated and exposed to fluorescent light overnight. The cells are assessed for viability by tritiated thymidine uptake as above.

For the B16G conjugates, A10, P815, and L1210 cells are used as target cells. (A10 cells are a T-cell hybridoma which secretes a B16G-reactive T suppressor factor; P815 cells are also reactive with B16G.) The in vitro study is done using a direct method employing the B16G-Hp or B16G-Gp conjugate or indirectly using unlabeled B16G antibodies and labeled RαMIg-Hp or RαMIg-Gp.

In a direct method, 5×10^5 cells are suspended in 1 ml DME/Hepes containing the appropriate Ig-drug conjugate as test or control at Hp or Gp concentrations of 320, 160, 80, 40 and 20 ng drug/ml. The cells are incubated in the dark at 37° for one hour, then washed three times in 5 ml DME/Hepes and then resuspended in 1 ml of the same buffer. Three 100 μl test portions of the labeled preparations are dispensed into flat bottom microtiter wells and the remainder of the cell suspensions (700 μl) are exposed to incandescent light (22.5 mW/cm^2) at a distance of 20 cm for one hour. Then three additional 100 μl aliquots are removed to microtiter wells. Tritium-labeled thymidine diluted in DME/-Hepes containing 20% FCS is then added to all microtiter wells in 100 μl aliquots so that 2 μCi of labeled thymidine is added to each well. Cultures are incubated for 18 hours at 37° C. and humidified 10% CO_2 and then harvested on a MASH harvester. Thymidine incorporation was measured with an Hp scintillation counter (Tri-Carb Model 4550). The results of this study for Ig-Hp are shown in Table 2.

TABLE 2

(ng Hp/ml)	% killing of cell lines A10	P815	L1210
B16G Hp			
320	100	70	55
160	100	50	10
80	100	20	0
40	65	10	0
20	20	0	0
C-Mab-Hp			
320	63	75	50
160	35	48	15
80	0	25	0
40	0	12	0
20	0	0	0

In an indirect assay, the A10 suspended cells, prepared as described above, are exposed to 50 μg/ml of either B16G or a control antibody C-Mab at 4° C. for 30 minutes, washed in DME/Hepes, and then exposed for an additional 30 minutes at 4° C. in the dark to varying concentrations of RαMIg-Hp or RαMIg-Gp between 2 μg/ml and 15 ng/ml of Hp or Gp. The cells are assessed for viability using labeled thymidine uptake as described above. These results for Ig-Hp are shown in Table 3.

TABLE 3

RαMIg-Hp (ng/ml)	Primary antibody B16G	C-MAb
500	100	30
250	85	22
125	75	5
52.5	60	2
31.2	47	3
15.6	18	1.5

Similar results are obtained using corresponding conjugates with Gp.

EXAMPLE 5

In Vivo Cytotoxicity of the Immunoconjugates

The efficacy of the conjugates and of the Gp compounds of the invention in vivo is also assessed. For the CAMAL-1 and anti-Ml conjugates, the procedures are as described in the two Mew, et al, papers referenced above in Example 4. The Gp compound alone shows superior results at appropriate wavelengths as compared to the Hp labeled conjugates.

For the B16G-Hp or B16G-Gp conjugates and for the Gp (BPD-DB) alone, the in vivo studies are conducted as follows:

The in vivo test relies on the indirect effect of a population of T-suppressor cells on tumors, which then serve as means to assess the effectiveness of the irradiation treatment. P815 mastocytoma cells grown in syngeneic DBA/2 mice stimulate T-suppressor cells specific for the tumor. These T-suppressor cells impede the development of specific T-killer cells which would otherwise aid in the regression of the tumor. The T-cell hybridoma designated A10 above secretes a T-suppressor factor which is associated with these T-suppressor cells. Thus, selective killing of these T-suppressor cell populations through reaction with conjugates in which the Ig is an antibody specific for the T-suppressor factor on the surface of the cells (namely B16G) should result in tumor regression in mice bearing the P815 tumors.

Therefore, in this assay, DBA/2 mice are injected in the right flank subcutaneously with 10^4 P815 cells to incorporate the tumor. On day eight, when the tumors are palpable approx. 25–42 sq mm) the mice are randomly sorted into groups of eight and injected IV with 150 μl PBS containing nothing, Hp or Gp, B16G-Hp or B16G-Gp, B16G plus either drug, B16G alone or C-MAbHp or C-MAb-Gp. The levels of Hp are 50 μg per animal in all cases and B16G 310 μg in all cases (where appropriate).

The animals are maintained in the dark for two hours and then exposed to strong light at 300–750 nm and 2.5 mW/cm^2 The animals were then treated normally and monitored on a daily basis.

Animals treated with B16G Hp survived and were tumor free after 100 days. Results obtained are shown in Table 4.

TABLE 4

	Experiment Treatment	Mean survival time (days)	No. of cures	% tumor-free after 100 days
1	PBS	25.0	0/7	0
	B16G-Hp	41.3	3/9	33
2	PBS	23.5	0/6	0
	Hp	21.0	0/8	0
	B16G-Hp	24.2	3/8	37.5
3	PBS	24.1	0/7	0
	Hp	23.4	0/7	0
	B16G + Hp	23.5	0/6	0
	B16G-Hp	29.2	2/7	29
4	PBS	25.2	0/8	0
	B16G	28.3	0/8	0
	Hp	24.2	0/8	0
	B16G + Hp	24.6	0/7	0
	B16G-Hp	36.7	3/7	43
5	PBS	23.8	0/8	0
	Hp	27.0	0/8	0
	C-MAb-Hp	20.3	0/8	0
	B16G-Hp	34.0	1/8	12.5

Similar results are obtained for Gp alone or Gp conjugates.

EXAMPLE 6

In Vitro Evaluation of BPD-DA, -MA, -DB and -MB

The four compounds shown in FIG. 2, wherein R^1 and R^2 are carbomethoxy, were tested in vitro as described in Example 1. All four compounds were photosensitive; the monoacid forms BPD-MA and BPD-MB were somewhat more active.

EXAMPLE 7

Biodistribution and Decradation

Biodistribution studies have been conducted using tritiated BPD-MA and BPD-MB. Table 5 shows the ratios between ^{3}H-BPD-MA concentration in the tumor and in normal tissues determined at various times post-injection in mice bearing P815 tumor as the average for 3 mice.

TABLE 5

Tissue	Time Post Injection 3 h	24 h	48 h	72 h	96 h	168 h
Blood	0.52	1.45	1.37	1.66	2.77	3.65
Brain	3.76	3.06	2.92	2.69	4.18	6.91
Heart	1.09	1.71	1.63	1.46	2.24	2.51
Intestine	2.42	1.85	1.88	1.48	3.29	2.23
Lung	0.79	1.55	1.47	1.16	1.63	1.79
Muscle	2.68	2.98	2.77	2.16	3.45	4.23
Skin	2.57	1.64	1.95	1.57	2.03	3.51

TABLE 5-continued

Tissue	Time Post Injection 3 h	24 h	48 h	72 h	96 h	168 h
Stomach	1.57	1.89	2.08	2.04	2.23	2.98

Tumor skin ratios are most favorable 3 hours after IV administration of the drug.

To determine biodegradability, tritiated BPD-MA was injected IV into P815 tumor-bearing mice. The mice were sacrificed at either 3 or 24 hours following injection and tumors, livers and kidneys were removed. The BPD-MA in these tissues was extracted and photoactivity was assessed in P815 target cells as described above in Example 1 under standard in vitro conditions. While 100% of BPD-MA in tumor was active at hours, only 39% was active at 24 hours; both the liver and kidney degraded BPD more rapidly than did tumor tissue. Administration of tritiated BPD-MB in the same system gave similar results.

Similar studies using BPD-MA conjugated to an anti-keratin Mab in a model murine system carrying the KLN squamous tumor cell line showed improved concentration of the drug in the target tissue.

EXAMPLE 8

In Vivo Photosensitization by BPD

Studies of potential photosensitizers were performed using the M-1 rhabdomycoscercoma system in DBA/J2 mice. The compositions to be tested were diluted to a concentration of 800 μg/ml in PBS from a stock 25 solution in DMSO at 8 mg/ml (except Photofrin ® II, which was diluted directly from the clinical vial). Animals (8 per group) received 0.1 ml (80 μg) of material IV 24 h prior to exposure to light, provided by a 150W tungsten bulb, red filter (transmits light >600 nm), hot mirror (reflects light >720 nm) and 2 fiber optics, at 567 Jo/cm^2.

The results, shown in Table 6, indicate all BPD compounds tested gave positive results. The superior results shown by Photofrin ® II compositions are explainable by the observation that initial tumor sizes were smaller (a result of chance).

TABLE 6

Photosensitizer	Days Tumor Free (PR)	Number of Cures*	Tumor Volume at Time of Light Treatment (mm^3)
None	0.5	2	22.4 ± 7.8
Photofrin ® II composition	21.3	5	11.9 ± 6.9
BPD-MA	9.2	4	19.0 ± 13.0
BPD-MB	10.6	3	18.2 ± 11.0
BPD-DA	10.7	4	18.7 ± 9.9
BPD-DB	10.6	3	25.4 ± 16.4

*Animals whose tumors regressed and who remained tumor-free for 30 days.

Similar studies, except using a light dose of 378 To/cm^3 resulted in the outcome shown in Table 7.

TABLE 7

Photosensitizer	Number of Animals	Days Tumour-free	Number of Cures
None	11	0.1	2
Photofrin II	10	9.5	4
BPD-MA	10	13.2	4
BPD-MB	9	8.7	6
BPD-DA	15	2.5	4
BPD-DB	13	13.0	8

The foregoing results are preliminary, and the assay protocols have not yet been optimized.

EXAMPLE 9

Alternate In Vivo Assay

Mice bearing small tumors were injected IV with drug to be tested. Three hours later the animals were sacrificed and their tumors removed. The tumor cells were teased apart to form a single cell suspension, and the cells were plated at 10^5/well and exposed to light at a prescribed dose. The plates were incubated overnight and assayed for viability by MTT assay.

The results of one study are shown in Table 8.

TABLE 8

Photosensitizer	Dose (μg/mouse)	Light Dose (Jo)	% Kill
BPD-MA	33	5.7	22.0
	40	3.8	32.5
	80	3.8	63.5 ± 2.1
	80	3.8	53.7 ± 6.2
BPD-MB	33	5.7	25.2
BPD-DA	80	3.8	11.0
	80	7.6	26.0

Thus, the BPD forms tested were active in this assay; it appears light intensity and drug levels are subject to optimization and correlation.

EXAMPLE 10

Comparison of BPD to Photofrin® II Compositions

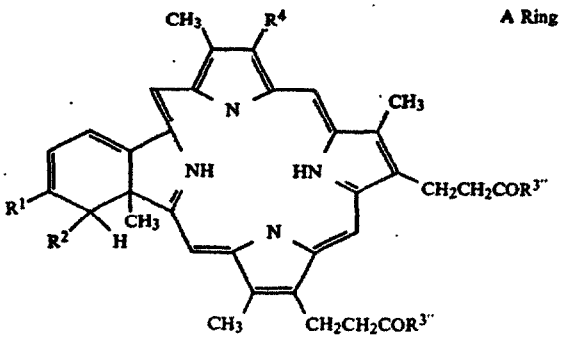
Mice bearing P815 tumors were shaved and injected with equivalent amounts of photosensitizer, and exposed to 72 Jo/cm^2 (80 mw/cm^2–15 min-full spectrum) at various time intervals. Skin biopsies were taken at 24 and 48 hours after light irradiation and visual evaluations were made blind. The results of these evaluations are shown in FIG. 4. BPD-MA and, to a lesser extent, BPD-MB had major photosensitizing activity, under these conditions; this was only present when light treatment was given 3 hours post drug administration, consistent with the biodegradability of these compounds.

EXAMPLE 11

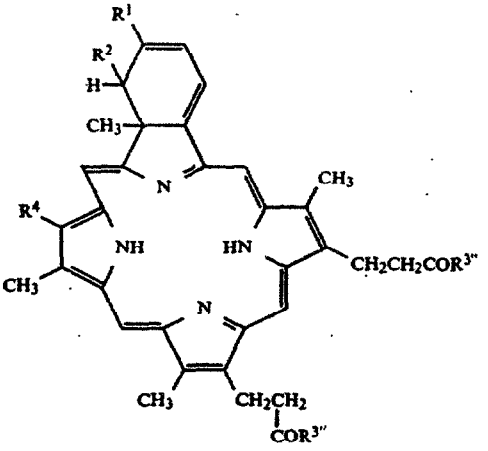
Preparation of Compounds of the Invention

The following compounds have been prepared using the above-described Diels-Alder reaction of MeOOC—C=C—COOMe with the dimethyl ester of protoporphyrin IX, followed by rearrangement to the forms shown as formulas 3 and 4 of FIG. 1 and by subsequent treatment to hydrolyze or modify the propionic ester on rings C and D and/or to modify the unreacted vinyl group on the A or B ring remaining after the Diels-Alder reaction with the B or A ring, as the case may be. The products are compounds of the following formulas, wherein $R^{3''}$ is OR* or NR* wherein R* is alkyl, alkylene, or H (or an organic or unorganic cation):

A Ring

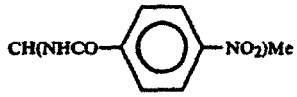
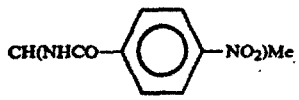


B Ring

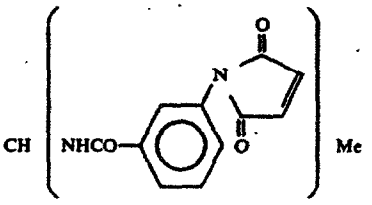
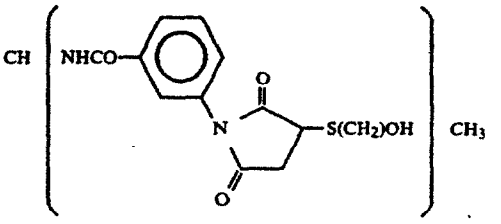


wherein R^1 and R^2 are, in all cases, COOMe.

The compounds prepared are as follows:

$R^{3''}$(C)	$R^{3''}$(D)	R^4
		A-Ring
1. OMe	OMe	$CHCH_2$
2. OH	OMe	$CHCH_2$(BPD-MA)
3. OMe	OH	$CHCH_2$(BPD-MA)
4. OH	OH	$CHCH_2$(BPD-DA)
5. OMe	OMe	$CH(NH_2)Me$
6. OMe	OMe	
7. OH	OH	
		B-Ring
1. OMe	OMe	$CHCH_2$
2. OH	OMe	$CHCH_2$

-continued

	$R^{3''}$(C)	$R^{3''}$(D)	R^4
3.	OMe	OH	$CHCH_2$
4.	OH	OH	$CHCH_2$
5.	OMe	OMe	$CH(NH_2)Me$
6.	OH	OH	$CH(NH_2)Me$
7.	OMe	OMe	$CH(NH(CH_2)_6NH_2)CH_3$
8.	OH	OH	$CH(NH(CH_2)_6NH_2)CH_3$
9.	OCD_3	OCD_3	$CH(NH(CH_2)_6NH_2)CH_3$
10.	OMe	OMe	CH(imidazolyl)CH_3
11.	OMe	OMe	
12.	OMe	OMe	
13.	OMe	OMe	CH(OH)Me
14.	OMe	OMe	CHBrMe
15.	OMe	OMe	CH(OMe)Me
16.	OMe	OMe	CH(pyridinium Br)Me
17.	$NH(CH_2)_6NH_2$	OMe	$CHCH_2$
18.	$R^{3''}$	$-R^{3''}-NH(CH_2)_6NH-$	$CHCH_2$
19.	OMe	OMe	$CH(SH)CH_3$
20.	OMe	OMe	disulfide of above
21.	OMe	OMe	CHO
22.	OMe	OMe	$CHOHCH_2OH$

EXAMPLE 12

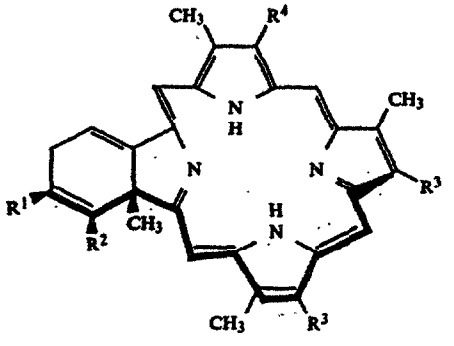
Preparation of BPD dimer-Vinyl Linked

To a stirring solution of BPD-DB (wherein R^1=R^2=carbomethoxy and which is esterified so that both R^3 are carbomethoxyethyl) (35 mg, 48 μmol) in 5 ml of dichloromethane cooled to dry ice/acetone temperature was added trifluoromethanesulfonic acid (34 μl, 380 μmol). An oil separated out upon the addition of the acid. The reaction was brought up to 0° C. Then 5 ml of 5% sodium bicarbonate was added to the reaction to neutralize the acid. The product distributed into the organic layer which was washed three times with water. The solvent was removed and the product was dried via azeotrope with acetonitrile.

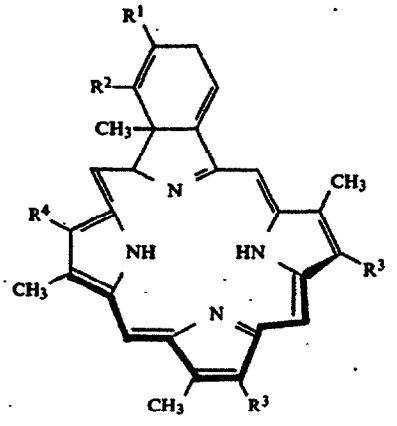
Preparative thin layer chromatography on silica gel eluting with 10% ethylacetate/dichloromethane gave a single fraction (28 mg, 80% yield). Parent ion in mass spectrum was 1464. The complex proton NMR due to the number of isomeric compounds had the characteristic single vinyl hydrogen associated with a C-linkage at about 8.1 ppm.

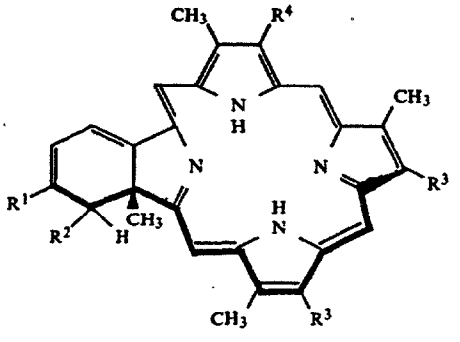
We claim:

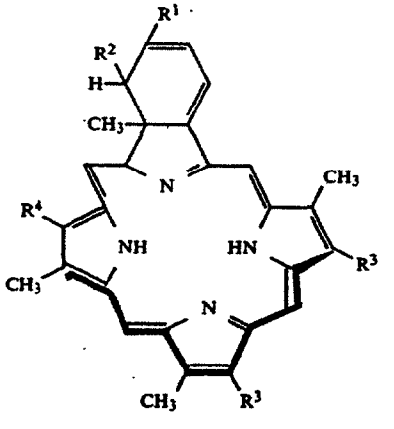
1. A compound of the formula

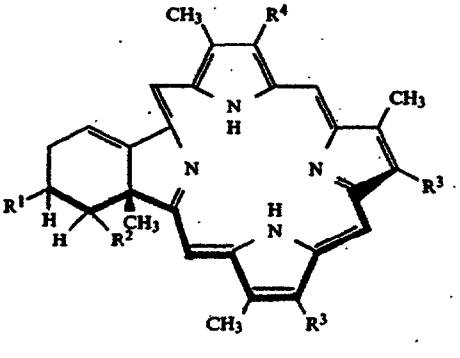


-continued



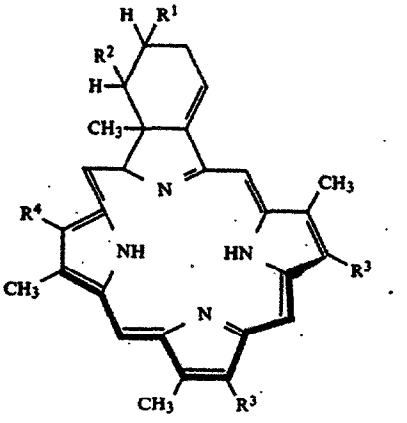






or

-continued



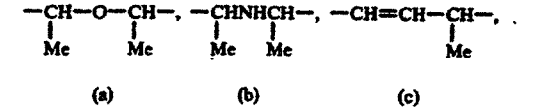
or the metalated and/or labeled form thereof;

wherein each R^1 and R^2 is independently selected from the group consisting of carbalkoxy (2-6C), aklyl (1-6C) sulfonyl, aryl (6-10C) sulfonyl, aryl (6-10C); cyano; and —$CONR^5CO$—where R^5 is aryl (6-10C) or alkyl (1-6C);

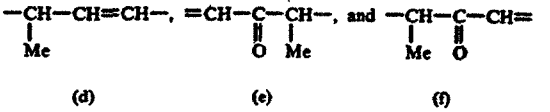
each R^3 is independently carboxyalkyl (2-6C) or a salt, amide, ester or acylhydrazone thereof, or is aklyl (1-6C); and

R^4 is $CHCH_2$, $[CHOR^{4'},]$—$CH_2OR^{4'}$—CHO, —$COOR^{4'}$, —$CH(OR^{4'})CH_3$,$CH(OR^{4'})CH_2OR^{4'}$,—$CH(SR^{4'})CH_3$, —$CH(NR^{4'}_2)$ CH_3, —$CH(CN)CH_3$, —$CH(COOR^{4'})CH_3$, —CH-$((OOCR^{4'})CH_3$, —CH(halo)CH_3, or —CH-(halo)CH_2(halo), wherein $R^{4'}$is H or alkyl (1-6C) optionally substituted with a hydrophilic substituent, or

wherein R^4 consists of 1-3 tetrapyrrole-type nuclei of the formula -L-P wherein -L- is selected from the group consisting of

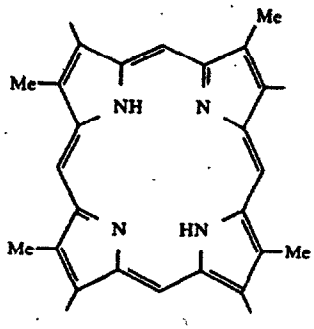


(a) (b) (c)



(d) (e) (f)

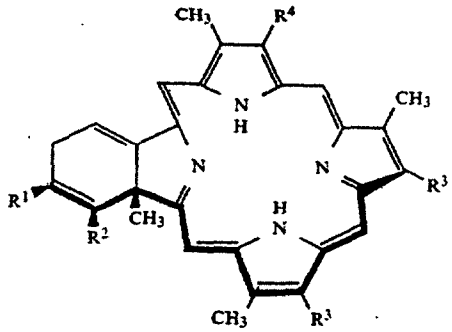
and P is selected from the group consisting of Gp which is of the formula 1-6 but lacking R^4 and conjugated through the position shown as occupied by R^4 to L, and a porphyrin of the formula:

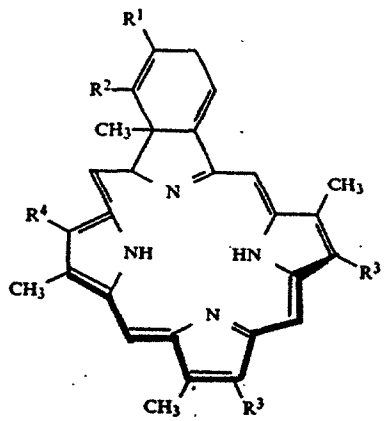


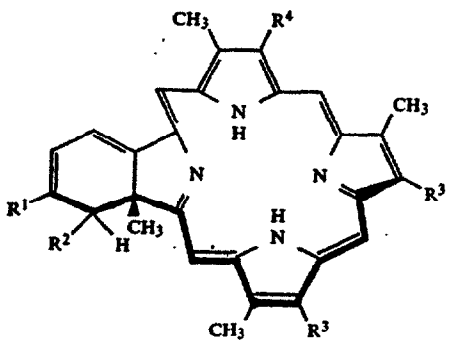
wherein two of the bonds shown as unoccupied on adjacent rings are joined to R^3 and one of the remaining bonds shown as unoccupied is joined to R^4 and the other to L;

with the proviso that if R^4 is $CHCH_2$, both R^3 cannot be carbalkoxyethyl.

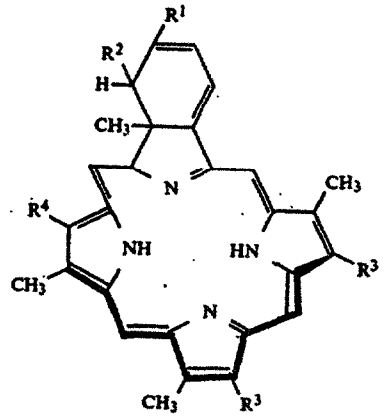
2. A compound of the formula

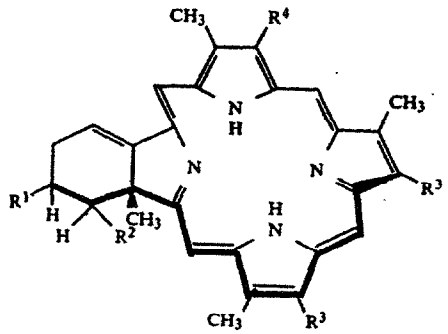




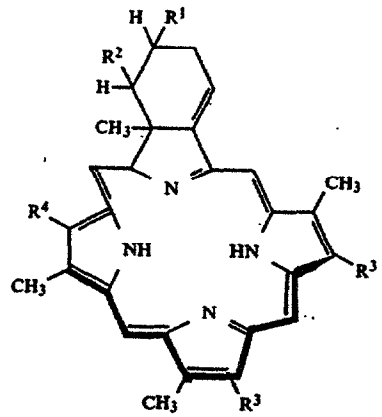


-continued





or



or the metalated and/or labeled form thereof;

wherein each R^1 and R^2 is independently selected from the group consisting of carbalkoxy (2–6C), alkyl (1–6C) sulfonyl, aryl (6–10C) sulfinyl, aryl (6–10C); cyano; and —$CONR^5CO$—where R^5 is aryl (6–10C) or alkyl (1–6C);

each R^3 is independently carboxyalkyl (2–6C) or a salt, amide, ester or acylhydrazone thereof, or is alkyl (1–6C); and

wherein R^4 is a non-interfering organic group of <12C resulting from direct or indirect derivatization of vinyl.

3. The compound of claim 1 or 2 wherein R^1 and R^2 are carbalkoxy.

4. The compound of claim 3 wherein R^3 and R^2 are carbomethoxy or carboxethoxy.

5. The compound of claim 1 or 2 wherein each R^3 is —CH_2CH_2COOH or a salt, amide, ester or acylhydrazone thereof.

6. The compound of claim 3 wherein each R^3 is —CH_2CH_2COOH or a salt, amide, ester or acylhydrazone thereof.

7. The compound of claim 1 or 2 which is of formulae 3 or 4.

8. The compound of claim 6 which is of formulae 3 or 4.

9. The compound of claim 1 or 2 wherein

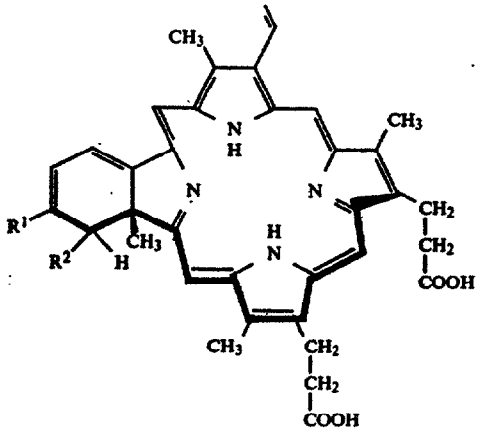
wherein R^4 is a group containing 1–3 tetrapyrrole-type nuclei of the formula -L-P.

10. The compound of claim 8 wherein

wherein R^4 is a group containing 1–3 tetrapyrrole-type nuclei of the formula -L-P.

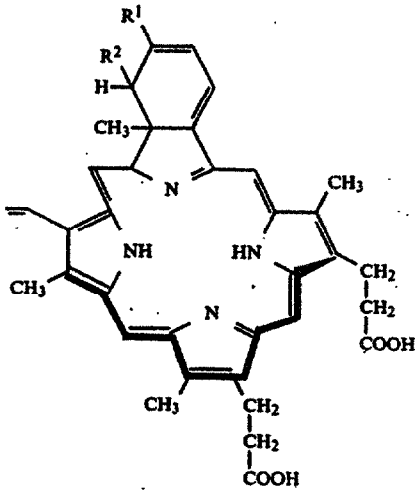
11. The compound of claim 1 or 2 which is selected from compounds of the formula

BPD-DA

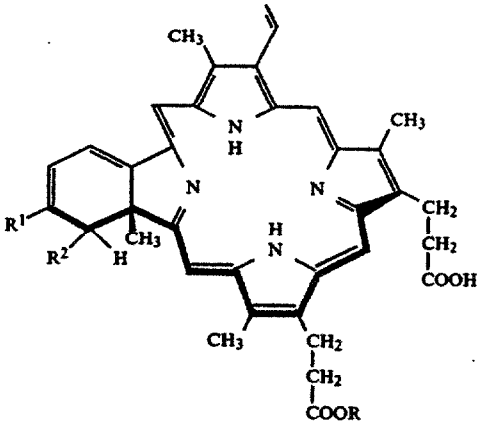


-continued

BPD-DB

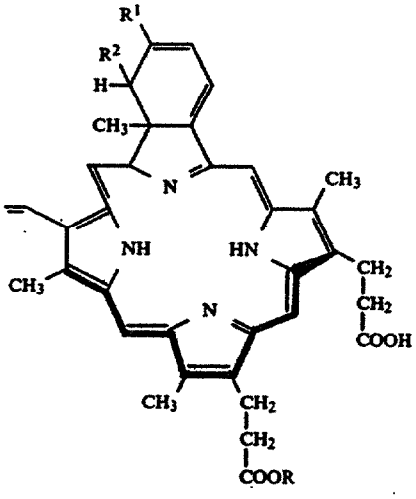


BPD-MA



and

BPD-MB



wherein R is alkyl (1–6C).

12. A pharmaceutical composition which is useful in targeting specific biological material which composition comprises an effective amount of the compound of claim 1 or 2 in admixture with at least one pharmaceutically acceptable excipient.

* * * * *

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:
Julia G. Levy, et al.
Serial No.: 07/414,201 Group Art Unit: 129
Filing Date: September 28, 1989 Examiner: R. Raymond
Title: WAVELENGTH-SPECIFIC CYTOTOXIC AGENTS

I hereby certify that this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 29 Jan. 1991
Date
Nancy Jane Thom
Signature
29 Jan. 1991
Date

TERMINAL DISCLAIMER

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

The University of British Columbia, having an address at Vancouver, British Columbia, Canada, is the owner of the entire right, title and interest in and to application U.S. Serial No. 07/414,201, filed 28 September 1989, by virtue of an assignment recorded on 26 October 1989 at Reel 5180, Frame 0357, and is also the owner of the entire right, title and interest in and to U.S. Patent No. 4,920,143, filed 19 July 1988 and issued 24 April 1990, by virtue of an assignment recorded on 6 September 1988 at Reel 4943, Frame 0021. The University of British Columbia hereby disclaims the terminal part of any patent granted on the herein application Serial No. 07/414,201 which would extend beyond the expiration date of U.S. Patent No. 4,920,143, and agrees that any patent granted on the herein application Serial No. 07/414,201 will be enforceable only for and during such period that the legal title to said patent shall be the same as the legal title to U.S. Patent No. 4,920,143.

Exxecuted at Vancouver, British Columbia, Canada on 10 January 1991.

By R. D. Spratley
Title R. D. SPRATLEY
DIRECTOR, RESEARCH SERVICES
THE UNIVERSITY OF B.C.

United States Patent and Trademark Office

USPTO Home Page

Finance Home Page

Maintenance Fee Statement

5095030

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 11, "STAT" below.

If a maintenance fee payment is defective, the reason is indicated by code in column 11, "STAT" below. TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

ITEM NBR	PATENT NUMBER	FEE CDE	FEE AMT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT
1	5,095,030	1553	3150	0	07/414,201	03/10/92	09/28/89	12	NO	PAID

197

ITEM NBR	ATTY DKT NUMBER		
1	7301000322		

EXHIBIT D
NDA

Correspondence Table of Contents

Volume 1

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
23-Mar-99	Regulatory Agency		E-mail	21-119	Response from Lori re NDA #/PhTox list.
03-May-99	QLT		NDA	21-119	Timeline update for NDA 21-119.
03-May-99	QLT		E-mail	21-119	Presubmission volume numbering suggestion.
03-May-99	Regulatory Agency		Telephone Contact	21-119	June 23rd is fine for FDA pre-NDA CMC meeting.
03-May-99	QLT		E-mail	21-119	Request for clarification on database format.
03-May-99	Regulatory Agency		E-mail	21-119	Response from Wiley: it dons not matter dBase or SAS.
05-May-99	Regulatory Agency		E-mail	21-119	Presubmission volume numbering.
05-May-99	QLT		E-mail	21-119	Volume numbering example for NDA 21-119.
10-May-99	QLT		E-mail	21-119	Questions re TOC & 356h.
12-May-99	Regulatory Agency		Telephone Contact	21-119	Lori left a message and wanted to discuss the suggestions for the TOC.
17-May-99	Regulatory Agency		E-mail	21-119	Response from Lori: the presubmission form is 356h.
17-May-99	QLT		E-mail	21-119	Question re presubmission form.
17-May-99	Regulatory Agency		Telephone Contact	21-119	Lori called back regarding volume numbering for the two presubmissions and the final NDA.
28-May-99	QLT		Telephone Contact	21-119	Misc NDA 21-119 issues.
02-Jun-99	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of our presubmission of section 5 (Pharmacology/Toxicology) for NDA 21-119 submitted May 28, 99.
03-Jun-99	Regulatory Agency		E-mail	21-119	Presubmission volume numbering.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 1

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
04-Jun-99	QLT		E-mail	21-119	Asked if the biopharm reviewer will need the electronic datasets from the primary pharmacokinetic studies.
08-Jun-99	Regulatory Agency		Telephone Contact	21-119	Question from the FDA re preNDA CMC meeting package.
11-Jun-99	Regulatory Agency		Telephone Contact	21-119	FDA biopharm reviewer requested access to PK data in Excel spreadsheet.
24-Jun-99	QLT		PMA	21-119	Question about bibliography section of a PMA.
08-Jul-99	Regulatory Agency		Fax	21-119	User fee ID# for NDA 21-119 is 3757.
08-Jul-99	QLT		Telephone Contact	21-119	User fee number for NDA 21-119.
09-Jul-99	Regulatory Agency		E-mail	21-119	Question re electronic databases for supportive dermatology studies and response from Lori Gorski.
13-Jul-99	Regulatory Agency		E-mail	21-119	Response to CRF question received - all withdrawals, regardless, should be submitted.
14-Jul-99	QLT		Telephone Contact	21-119	Inquiry information on electronic submissions for NDA 21-119.
14-Jul-99	QLT		Telephone Contact	21-119	Spoke with Mike Roosevelt of the FDA Office of Financial Management to confirm the User Fee noted in the Dec/98 FR.
19-Jul-99	QLT		E-mail	21-119	Asking whether the FDA is able to accept and archive databases in SAS System XPORT transport format or data sets created directly on Window SAS.
19-Jul-99	QLT		Telephone Contact	21-119	Concern that if the NDA is later than mid August then our advisory meeting may not be until the new year. They also need 2 weeks notice of a press release.
20-Jul-99	QLT		E-mail	21-119	Lori's response to the question of which dataset format is preferred.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 1

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
22-Jul-99	QLT		E-mail	21-119	Confirmation of FDA address for submission.
29-Jul-99	QLT		E-mail	21-119	Asking what colour of covers should be used for the PMA Submission. Also advising Lori of our email revision in process.
30-Jul-99	QLT		Telephone Contact	21-119	Lori requests 2 copies of the PMA, one in blue and one in generic (non FDA) covers. Confirmation still needed from CDRH if one copy will be adequate.
30-Jul-99	QLT		E-mail	21-119	Response from Lori stating they only need one set of photographs in blue jackets labeled as archival. PMA information to follow.
02-Aug-99	QLT		Telephone Contact	21-119	Received telephone call from Lori Gorski about the PMA submission.
04-Aug-99	QLT		E-mail	21-119	Received email from Lori regarding the device submission and how it is to be submitted.
09-Aug-99	QLT		Telephone Contact	21-119	Received a telephone call from Richard Felten about an earlier question concerning bibliography of the PMA.
09-Aug-99	QLT		Telephone Contact	21-119	Spoke with Lori Gorski concerning PMA submission.
10-Aug-99	QLT		E-mail	21-119	E-mail from Lori on the timing for the NDA.
11-Aug-99	QLT		E-mail	21-119	Informing Richard that there will be a CD-ROM in the desk copy of Volume 1.
11-Aug-99	QLT		E-mail	21-119	Informing Lori of which volumes contain the angiograms and photos. They are volumes 245-250 inclusive.
11-Aug-99	QLT		E-mail	21-119	Sent an email to Richard Felten letting him know that the PMAs will be sent directly to CDRH.
11-Aug-99	QLT		NDA	21-119	FDA Form 356h sent to Jonathan for signature. Informing him of the number of volumes to be submitted.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 1

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
12-Aug-99	QLT		Telephone Contact	21-119	Lori called asking if we were going to the AAO from Oct. 24-27 and who she would speak to on the advisory committee.
12-Aug-99	QLT		E-mail	21-119	Richard Felten wants only 1 copy of volume 1 of the NDA.
12-Aug-99	QLT		E-mail	21-119	Discussions with Richard about the submission date arrival and the deskcopy of volume 1.
12-Aug-99	QLT		E-mail	21-119	Letting Richard know that the electronic copy of the PMAs might be helpful to him. They are also unofficially in the desk copy of volume 1 of the NDA.
12-Aug-99	QLT		Telephone Contact	21-119	Lori mentioned that our User Fees are listed as paid.
13-Aug-99	QLT		Telephone Contact	21-119	Asking Lori where to send the field copy of the CMC.
13-Aug-99	QLT		Fax	21-119	Faxed Lori a copy of the press release for Monday, August 16, 1999.
17-Aug-99	QLT		Telephone Contact	21-119	Received voicemail from Dr. Kadar who had some questions concerning the NDA.
19-Aug-99	QLT		Telephone Contact	21-119	Lori called stating that there are some things missing from the NDA that would result in the application being refused.
19-Aug-99	QLT		Telephone Contact	21-119	Agreement with Gary Pierce on where to send field copies of NDA.
20-Aug-99	QLT		Acknowledgement	21-119	Acknowledgement of receipt of NDA 21-119.
20-Aug-99	QLT		Fax	21-119	Acknowledgement of receipt of NDA 21-119 Submission dated August 14, 1999.
20-Aug-99	QLT		Request	21-119	Submitted to FDA an Amendment to a Pending Application - Request for Waiver from Pediatric Studies.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 1

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
20-Aug-99	QLT		Telephone Contact	21-119	Lori requests 4 more desk copies of volume 1.
23-Aug-99	QLT		Telephone Contact	21-119	Tony requests the names of the pivotal studies for NDA 21-119, the centers and the number of patients enrolled.
23-Aug-99	QLT		General Correspondence	21-119	Faxed Lori a copy of the August 24 press release - VISUDYNE Application For AMD Granted Priority Review by FDA.
23-Aug-99	QLT		General Correspondence	21-119	Submitted an additional four copies of volume 1 of NDA 21-119 to Lori as requested.
23-Aug-99	QLT		General Correspondence	21-119	Submitted the information requested for the pivotal studies BPD OCR 002A and B.
23-Aug-99	QLT		Fax	21-119	Acknowledgement of receipt of request for waiver to perform clinical investigations in pediatric populations.
25-Aug-99	Regulatory Agency		Telephone Contact	21-119	Potential date for Advisory Committee meeting.
25-Aug-99	QLT		Telephone Contact	21-119	Lori called back with information on the Advisory meeting date.
26-Aug-99	QLT		Telephone Contact	21-119	Received voicemail from Carol stating that she required some additional information on the NDA as well as some clarification.
01-Sep-99	QLT		Telephone Contact	21-119	Discussions with Lori about the Advisory committee contact, Alex's resignation and the four missing desk copies of volume 1 of the NDA.
01-Sep-99	QLT		E-mail	21-119	Giving Lori the confirmation of delivery information of the four missing deskcopies of volume 1 of the NDA. Lori's phone message letting us know she found them.
01-Sep-99	QLT		E-mail	21-119	Informing Lori of a press release for TIND going out on September 13th.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 1

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
03-Sep-99	QLT		E-mail	21-119	Some follow-up information re Larry's role here at QLT.
03-Sep-99	QLT		E-mail	21-119	Lori needs a letter stating that Larry is now the contact for the NDA.
07-Sep-99	QLT		Telephone Contact	21-119	Left voicemail with Tracy Riley to introduce myself, our NDA and our product.
08-Sep-99	QLT		NDA	21-119	Amendment to a Pending Application stating Larry Mandt has assumed Alex's responsibilities.
08-Sep-99	QLT		Fax	21-119	Faxed Lori a copy of the amendment to NDA 21-119 that was submitted today.
08-Sep-99	QLT		Fax	21-119	Faxed Richard a copy of the cover letter that was submitted to FDA for the Amendment to a pending application.
10-Sep-99	QLT		Fax	21-119	Acknowledgement of receipt of amendment to a pending application dated September 8, 1999.
13-Sep-99	Regulatory Agency		Telephone Contact	21-119	Tony Carreras called to inform us which clinical sites would be inspected and to request information.
13-Sep-99	QLT		Telephone Contact	21-119	Raylo received a call concerning an inspection for the TIND.
15-Sep-99	Regulatory Agency		Fax	21-119	Lori has some comments/requests from the chemistry reviewer that need to be addressed.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 2

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
16-Sep-99	Regulatory Agency		Fax	21-119	Received faxed letter granting us a waiver for pediatric studies.
22-Sep-99	Regulatory Agency		Telephone Contact	21-119	Received a call from Tracy Riley re details related to the pending Advisory Panel for VISUDYNE confirmed for November 17, 1999 from 8:30 to 5:00.
22-Sep-99	Regulatory Agency		Fax	21-119	Received fax from Lori containing comments pertaining to VISUDYNE.
23-Sep-99	QLT		Amendment	21-119	Faxed Lori a copy of the cover letter of the CMC Amendment submitted to FDA today.
23-Sep-99	QLT		Amendment	21-119	Submitted three copies of CMC Amendment to FDA as well as the response to the Chemistry Reviewer questions.
24-Sep-99	QLT		Telephone Contact	21-119	Enquiring whether or not the FDA investigator would need a copy of the CMC amendment I sent to FDA.
27-Sep-99	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of CMC Amendment submitted to FDA September 23, 1999.
28-Sep-99	Regulatory Agency		Telephone Contact	21-119	Received a call from Lori stating she required 3 copies of the CMC amendment (which we sent) in the appropriate colour jackets.

Correspondence Table of Contents

Volume 3

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
04-Oct-99	QLT		E-mail	21-119	This response is in reference to your query on the EFFICACY.SD2 electronic database relating to study BPD OCR 002A.
05-Oct-99	Regulatory Agency		Fax	21-119	Received a fax from Lori with comments/questions from the chemistry reveiwer for submission of NDA 21-119. Request response with an amendment.
06-Oct-99	QLT		Amendment	21-119	Submitted an Amendment to a Pending Application (Electronic Database) in response in reference to a query discussed during a teleconference concerning the EFFICACY.SD2 electronic database relating to Study BPD OCR 002A.
06-Oct-99	QLT		Information Amendment	21-119	Submitted additional Microbiology information to FDA, further to a telephone request from Dr. Carol Vincent.
07-Oct-99	QLT		Fax	21-119	Faxed Lori a copy of the cover letter that was submitted to FDA with additional Microbiology information, further to a telephone request from Dr. Carol Vincent.
07-Oct-99	QLT		Fax	21-119	Faxed Richard a copy of the cover letter of an Amendment to a Pending Application (Electronic Database) that was submitted to FDA October 6, 1999.
08-Oct-99	QLT		Telephone Contact	21-119	Spoke with Dr. Carreras about the information we were to send him for the clinical audits.
12-Oct-99	QLT		Telephone Contact	21-119	Left voicemail re inspection.
12-Oct-99	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of additional Microbiology information submitted October 6, 1999.
12-Oct-99	QLT		Telephone Contact	21-119	Telephone conversation with Pat re inspections for sites.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 3

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
12-Oct-99	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of Amendment to a Pending Application (Electronic Database) submitted October 6, 1999.
12-Oct-99	QLT		Amendment	21-119	Submitted additional pharmacokinetics information requested by the Agency as an amendment to a Pending Application
12-Oct-99	QLT		Amendment	21-119	Submitted review and archival copies of our response to pharmacology/toxicology comments received from FDA on Sept. 22/99.
12-Oct-99	QLT		Fax	21-119	Faxed Richard a copy of the cover letters for Amendment to a Pending Application (Pharmacology/Toxicology) and (Pharmacokinetics) submitted to FDA today.
12-Oct-99	QLT		Telephone Contact	21-119	Left a voicemail re scheduled inspections for NDA 21-119 for VISUDYNE.
12-Oct-99	Regulatory Agency		Telephone Contact	21-119	Received a call from Lori requesting further CMC information.

Correspondence Table of Contents

Volume 4

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
13-Oct-99	Regulatory Agency		Fax	21-119	Further requests from the chemistry reviewer. Respond with an amendment.
13-Oct-99	QLT		E-mail	21-119	Sent an email to Lori asking how many copies and what colour jackets are to be used in the next amendment.
13-Oct-99	Regulatory Agency		E-mail	21-119	Received an email from Lori re jacket colours for the labeling amendment.
14-Oct-99	Regulatory Agency		Telephone Contact	21-119	Dr. Carreras called asking for the name of the Principle Investigator and the site address for the Baltimore and Miami sites.
14-Oct-99	Regulatory Agency		Fax	21-119	Another request from the chemistry reviewer for further information and clarification of material.
14-Oct-99	QLT		Amendment	21-119	Submitted three copies of an Amendment (Labeling - Production prints for vial and box) to FDA today.
14-Oct-99	QLT		Fax	21-119	Faxed Richard a copy of the cover letter of an amendment to the labeling information that was submitted to FDA today.
14-Oct-99	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of Amendment to a Pending Apllication - Pharmacology/toxicology, and of Amendment to a Pending Application - Pharmacokinetics both submitted to FDA October 12, 1999.
18-Oct-99	QLT		Fax	21-119	Faxed Lori the reply to her fax of October 12, 1999 re chemistry reviewer questions.
18-Oct-99	QLT		General Correspondence	21-119	A copy of the cover letter sent to Tracy Riley with twenty copies of the briefing document for the November 17, 1999 Advisory Committee meeting.
18-Oct-99	QLT		General Correspondence	21-119	A copy of the cover letter to Lori Gorski with twelve copies of the briefing document for the November 17, 1999 Advisory Committee meeting.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 4

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
18-Oct-99	QLT		Amendment	21-119	Submitted in triplicate the breifing document for the November 17, 1999 Advisory Committee Meeting.
18-Oct-99	QLT		Amendment	21-119	Submitted a CMC amendment as well as the response to the chemistry reviewer questions of Oct. 12, 1999.
18-Oct-99	QLT		Fax	21-119	Faxed Richard a copy of the cover letter of the Amendment (Advisory Committee Briefing Document) sent to FDA today.
18-Oct-99	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of Amendment - Labeling - Production Prints for vial and box submitted to FDA October 14, 1999.
18-Oct-99	QLT		Fax	21-119	Faxed Lori a copy of the Amendment to a Pending Application (Pharmacokinetics) submitted Oct. 12, 1999.
20-Oct-99	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of CMC amendment submitted Oct 18/99.
20-Oct-99	Regulatory Agency		Fax	21-119	Fax from Lori outlining items from the pharmacology/toxicology reviewer that need clarification/information. Respond with an amendment.
20-Oct-99	Regulatory Agency		Telephone Contact	21-119	Dr. Alan Fenselau expressed frustration in not receiving any response from Harimex on the questions brought forth to them.
20-Oct-99	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of Advisory Committee Briefing Document submitted Oct 18/99.
21-Oct-99	Regulatory Agency		Telephone Contact	21-119	The PK reviewer would like an electronic copy of the PK study, if we have it, submitted to her. Lori also suggested that we send three copies of any amendment to the FDA.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 4

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
21-Oct-99	Regulatory Agency		Fax	21-119	A request for clarification of material from Allan Fenselau. Respond with an amendment addressed to Lori Gorski before December 01, 1999.
21-Oct-99	QLT		E-mail	21-119	Lori asks that we always send three copies of any amendment. I also asked her what colour the cover should be for the third copy.
21-Oct-99	Regulatory Agency		E-mail	21-119	Lori's response to the jacket colour for amendment submissions.
21-Oct-99	QLT		Amendment	21-119	Submitted a single unofficial deskcopy (electronic version) of the pharmacokinetic information submitted in our NDA.
22-Oct-99	Raylo Chemicals Inc.		General Correspondence	21-119	re: inspection of Raylo by Margaret Smithers on Sept. 21-21 and Sept. 28-30, 1999. Raylo's response to the observations listed on the Forms FDA 483 dated September 24 and 30, 1999.

Correspondence Table of Contents

Volume 5

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
26-Oct-99	QLT		Fax	21-119	Faxed Allan a copy of the cover letter to the CMC amendment that was submitted to FDA today.
26-Oct-99	QLT		Fax	21-119	Faxed Richard a copy of the cover letter to the CMC amandment submitted to FDA today.
26-Oct-99	QLT		Fax	21-119	Faxed Lori a copy of the cover letter to the CMC amendment submitted to FDA today.
26-Oct-99	QLT		Amendment	21-119	Submitted response to reviewer questions from fax dated September 22, 1999.
27-Oct-99	QLT		Fax	21-119	Faxed Lori a copy of CMC amendment "Response to Reviewer Questions" submitted Oct 26/99 for Allan Fenselau.
27-Oct-99	Regulatory Agency		Telephone Contact	21-119	Voicemail from Allan stating to fax him a copy of the amendment asap.
28-Oct-99	Regulatory Agency		Fax	21-119	Allan requests a copy of VER-QLT-ANN-0007.
28-Oct-99	QLT		Briefing Document	21-119	Submitted one unofficial copy of the redacted advisory committee briefing document.
29-Oct-99	Regulatory Agency		Fax	21-119	Allan requests further information. Fax response to Lori by Wednesday Nov 3/99.
01-Nov-99	QLT		Fax	21-119	Faxed Allan a copy of the amendment submitted to FDA today.
01-Nov-99	Regulatory Agency		Fax	21-119	Allan requests copies of the Certificates of Analysis for the presomal ingedients DMPC, EPG, AP, and BHT- Lot no. 990413.
01-Nov-99	QLT		Amendment	21-119	Submitted a copy of the report VER-QLT-ANN-0007 as well as Impurity Profiles of Intermediates and API manufactured from Sato and Raylo PRIX-DME.
03-Nov-99	QLT		Amendment	21-119	Submitted a response to faxes received October 29/99 and November 01/99 from the Review Chemist.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 5

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
03-Nov-99	QLT		Fax	21-119	Faxed Allan a copy of the amendment that was submitted to FDA today.
03-Nov-99	QLT		Fax	21-119	Faxed Lori a copy of the cover letter of the amendment submitted to FDA today.
04-Nov-99	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of CMC amendment submitted November 3/99.
04-Nov-99	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of CMC Amendment dated November 1/99.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 6

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
05-Nov-99	Regulatory Agency		E-mail	21-119	Valentin asking for further info re Sato Pharmaceutical.
05-Nov-99	Regulatory Agency		Fax	21-119	Received a fax from FDA wanting to schedule inspections for Nippon and Sato. Asking for contact persons and fax numbers to make arrangements.
05-Nov-99	QLT		Fax	21-119	Faxed Lori a copy of the fax received re FDA inspections on Nippon and Sato.
05-Nov-99	QLT		Fax	21-119	Faxed Lourdes the information she requested to make arrangements for inspections.
05-Nov-99	Regulatory Agency		Fax	21-119	Further requests from Allan for more information. Fax responses to Lori by Thursday Nov. 11/99.
05-Nov-99	QLT		Telephone Contact	21-119	Discussions with Lori re copies of amendments and inspections of Nippon and Sato.
05-Nov-99	Regulatory Agency		Fax	21-119	Received a faxed copy of the draft label (PI) and questions for discussion at the advisory meeting.
05-Nov-99	QLT		Amendment	21-119	Submitted an amendment to authorize FDA to share information in NDA 21-119 with the BPA.
05-Nov-99	QLT		General Correspondence	21-119	Sent the November 17/99 Advisory Committee meeting agenda for the sponser plus a listing of investigators who participated in the pivotal Phase 3 studies.
05-Nov-99	QLT		Fax	21-119	Faxed Richard a copy of the cover letter submitted to FDA today.
05-Nov-99	Regulatory Agency		Telephone Contact	21-119	Lori left a message re the draft labelling and questions to be posed at the Advisory Meeting.
05-Nov-99	Regulatory Agency		E-mail	21-119	Response to Valentin re inspections of Sato.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 6

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
08-Nov-99	Regulatory Agency		Fax	21-119	Further requests from the chemistry reviewer. Fax response to Lori Gorski by Friday Nov. 12/99.
08-Nov-99	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of "Modified FDA Format" amendment submitted November 5/99.
09-Nov-99	QLT		Amendment	21-119	Submitted response to faxes received on October 5, 13, 14, and 21, 1999 with questions from the Review Chemist.
09-Nov-99	QLT		General Correspondence	21-119	Sent a copy of the response to Margaret of the Review Chemist's questions dated September 22, 1999.
09-Nov-99	QLT		E-mail	21-119	E-mailed Lori the list of advisory panel members.
09-Nov-99	QLT		E-mail	21-119	Asked Tracy if it was possible to videotape at the Advisory Committee Meeting.
09-Nov-99	Regulatory Agency		Telephone Contact	21-119	Lori called re possible chemistry issues at the Advisory Committee meeting and wondered if I was going to be there.
09-Nov-99	Regulatory Agency		Telephone Contact	21-119	Tracy Riley responds to question concerning video cameras at Advisory Meeting.
09-Nov-99	QLT		Fax	21-119	Faxed Tracy a revised copy of the agenda for the Nov. 17 panel meeting.

Correspondence Table of Contents

Volume 7

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
10-Nov-99	Regulatory Agency		Fax	21-119	Received by fax a draft assessment report from the Danish Medicine Agency and questions that might give us additional ideas re possible concerns of the Advisory Committee.
10-Nov-99	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of CMC amendment dated Nov. 9/99/
10-Nov-99	QLT		Fax	21-119	Faxed Lori a copy of the cover letter of the CMC amendment submitted to FDA today.
10-Nov-99	QLT		Fax	21-119	Faxed Allan a copy of the cover letter of the CMC amendment submitted to FDA today.
10-Nov-99	QLT		Amendment	21-119	Submitted the response to faxes received on October 5, 13, 21, and November 5, 8, 10, 1999 with questions from the review chemist.
10-Nov-99	Regulatory Agency		Fax	21-119	A request from Allan to provide copies of the verteporfin HPLC assays used to analyse verteporfin by the 60/40 and the 80/20 gradient system.

Correspondence Table of Contents

Volume 8

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
15-Nov-99	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of CMC amendment dated November 10, 1999.
15-Nov-99	QLT		Fax	21-119	Faxed Tracy a revised agenda for the Nov. 17 meeting.
17-Nov-99	Regulatory Agency	106	Acknowledgement	21-119	Acknowledgement of receipt of follow-up written safety report submitted Nov. 15/99.
19-Nov-99	Regulatory Agency		Fax	21-119	Requests from the pharmacology/toxicology reviewer. Respond with amendment.
23-Nov-99	QLT		Telephone Contact	21-119	Dr Ng, Dr. Allan Fenselau and Lori Gorski called re dioxin contaminated product.
23-Nov-99	Regulatory Agency		Fax	21-119	Received a fax from Allan asking for a statement to the effect that the lots of hemin used in the manufacture of VISUDYNE were not contaminated with dioxin.
24-Nov-99	QLT		Fax	21-119	Faxed Allan a copy of the CMC amendment submitted to FDA today.
24-Nov-99	QLT		Fax	21-119	Faxed Jonathan a copy of the cover letter of the CMC amendment submitted to FDA today.
24-Nov-99	QLT		Fax	21-119	Faxed Lori a copy of the cover letter of the CMC amendment submitted to FDA today.
24-Nov-99	QLT		Amendment	21-119	Submitted our response to the fax received on November 23, 1999 with questions from the review chemist.
26-Nov-99	QLT		Amendment	21-119	Submitted an amendment "revisions to labeling - package insert" to FDA.

Correspondence Table of Contents

Volume 9

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
29-Nov-99	QLT		Fax	21-119	Faxed Allan a copy of the response to the fax dated Oct. 05/99 that was submitted to FDA today.
29-Nov-99	QLT		Fax	21-119	Faxed Lori a copy of the cover letter that was submitted to FDA today.
29-Nov-99	QLT		Fax	21-119	Faxed Richard an amendment to the drug labeling proposed by CDER on Nov. 05/99.
29-Nov-99	QLT		Amendment	21-119	Submitted a CMC amendment in response to a fax received Oct. 05/99 from the Review Chemist.
29-Nov-99	QLT		Fax	21-119	As requested, we faxed Allan a copy of the Nov. 04/99 letter from Les Sudbury of ICN regarding anti-bovine serum.
29-Nov-99	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of CMC amendment submitted to FDA Nov. 24/99.
29-Nov-99	QLT		Information Amendment	21-119	Submitted our response to the pharmacology/toxicology comments received by fax on Nov. 19, 1999.
29-Nov-99	QLT		E-mail	21-119	E-mailed Lori Gorski and Wiley Chambers the electronic version of the labeling amendment.
30-Nov-99	QLT		Fax	21-119	Faxed Carole Vincent a copy of the letter of authorization from Parkedale Pharmaceuticals Inc.
30-Nov-99	Regulatory Agency			21-119	Acknowledgement of receipt of CMC amendment submitted to FDA Nov. 29/99.
30-Nov-99	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of revisions to lebeling -package insert submitted to FDA Nov.26/99.
30-Nov-99	QLT		Information Amendment	21-119	Submitted our response to the pharmacology/toxicology comments received by fax on October 20, 1999.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 9

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
30-Nov-99	Regulatory Agency		Telephone Contact	21-119	Carol Vincent called needing some microbiology information in order to recommend approval.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 11

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Information Amendment	21-119	Submitted a 12 volume 4-month Safety Update as information amendment. (Volume 2 of 12).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 12

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Information Amendment	21-119	Submitted a 12 volume 4-month Safety Update as information amendment. (Volume 3 of 12).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 13

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Information Amendment	21-119	Submitted a 12 volume 4-month Safety Update as information amendment. (Volume 4 of 12).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 14

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Information Amendment	21-119	Submitted a 12 volume 4-month Safety Update as information amendment. (Volume 5 of 12).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 15

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Information Amendment	21-119	Submitted a 12 volume 4-month Safety Update as information amendment. (Volume 6 of 12).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 16

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Information Amendment	21-119	Submitted a 12 volume 4-month Safety Update as information amendment. (Volume 7 of 12).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 17

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Information Amendment	21-119	Submitted a 12 volume 4-month Safety Update as information amendment. (Volume 8 of 12).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 18

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Information Amendment	21-119	Submitted a 12 volume 4-month Safety Update as information amendment. (Volume 9 of 12).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 19

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Information Amendment	21-119	Submitted a 12 volume 4-month Safety Update as information amendment. (Volume 10 of 12).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 20

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Information Amendment	21-119	Submitted a 12 volume 4-month Safety Update as information amendment. (Volume 11 of 12).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 21

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Information Amendment	21-119	Submitted a 12 volume 4-month Safety Update as information amendment. (Volume 12 of 12).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 22

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Dec-99	QLT		Fax	21-119	Faxed Jonathan a copy of the cover letter that was submitted to FDA today.
01-Dec-99	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of Information Amendment Pharmacology/Toxicology submitted to FDA on Nov. 29/99.
01-Dec-99	QLT		E-mail	21-119	Emailed Carole Vincent re information requested from Parkedale is not in their DMF.
01-Dec-99	QLT		Fax	21-119	Faxed Richard a copy of the cover letter that was submitted to FDA today.
02-Dec-99	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of information amendment - Pharmacology/Toxicology submitted Nov. 30/99.
02-Dec-99	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of information amendment - 4-month Safety Update submitted Dec. 01/99.
07-Dec-99	QLT		Fax	21-119	Faxed Lori our feedback on the Nov. 17/99 Ophthalmic Subcommittee Meeting.
08-Dec-99	QLT		Fax	21-119	A hard copy of the fax sent to the FDA re Advisory Panel Feedback.
08-Dec-99	Regulatory Agency		Telephone Contact	21-119	Voicemail from Lori re sending Wiley's slides and a pharmacology/toxicology reviewer comments.
09-Dec-99	QLT		Telephone Contact	21-119	Spoke with Irma re FDA inspection of QLT.
09-Dec-99	QLT		E-mail	21-119	Emailed Lori re VISUDYNE production prints for vial and box amendment sent Oct. 14/99, received in document room Oct. 18/99.
09-Dec-99	Regulatory Agency		Fax	21-119	Received a fax from FDA re inspection of QLT.
09-Dec-99	Regulatory Agency		Fax	21-119	Request from the pharmacology/toxicology reviewer.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 22

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
10-Dec-99	QLT		Information Amendment	21-119	Submitted our response to the pharmacology/toxicology comments received by fax Dec. 09/99.
10-Dec-99	Regulatory Agency		Telephone Contact	21-119	Confirmation of the address to send the desk copy of the Parkedale information to.
10-Dec-99	Regulatory Agency		Fax	21-119	FDA inspection of QLT confirmed to take place Feb. 04/2000.
10-Dec-99	QLT		Telephone Contact	21-119	Left message with Rochelle re inspection date for QLT of Feb. 04/00.
10-Dec-99	Regulatory Agency		Fax	21-119	Address to which desk copies of validation information is to be sent.
13-Dec-99	QLT		Telephone Contact	21-119	Spoke with Wiley re Advisory Panel
13-Dec-99	Regulatory Agency		Fax	21-119	Information on FDA Inspection team re arrival time, names, departure dates.
13-Dec-99	QLT		Telephone Contact	21-119	Spoke with Lori re inspection and approval letter.
13-Dec-99	QLT		Telephone Contact	21-119	Spoke with Lori re labeling, DDMAC review, and NDA reviews.
14-Dec-99	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of pharmacology/toxicology comments submitted Dec. 10/99.
14-Dec-99	QLT		Fax	21-119	Fax to Rochelle re hotel accommodation for FDA inspection team and confirmation of date of inspection.
14-Dec-99	Regulatory Agency		General Correspondence	21-119	Received a copy of the Advisory Committee Transcripts and the FDA Presentation Slides.

Correspondence Table of Contents

Volume 23

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
14-Dec-99	QLT		Information Amendment	21-119	Submitted CMC/Microbiology information containing requalification documentation for equipment used to manufacture VISUDYNE at Parkedale Pharmaceuticals. Vol. 1 of 2.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 24

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
14-Dec-99	QLT		Information Amendment	21-119	Submitted CMC/Microbiology information containing requalification documentation for equipment used to manufacture VISUDYNE at Parkedale Pharmaceuticals. Vol. 2 of 2.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 25

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
15-Dec-99	QLT		Telephone Contact	21-119	Left a message with Rochelle re inspection of QLT.
15-Dec-99	QLT		Telephone Contact	21-119	Left message with Rochelle re no microbiologist was necessary for QLT inspection.
17-Dec-99	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of Information amendment submitted Dec. 14/99.
22-Dec-99	Regulatory Agency		E-mail	21-119	Lori called re no new info at this time. Working on an approvable letter.
05-Jan-00	QLT		Telephone Contact	21-119	Spoke with FDA re status of the review for the VISUDYNE NDA. No new info expected until Jan. 17/00. Lori is going down to part time status (not working Tuesdays or Thursdays).
06-Jan-00	Regulatory Agency		Fax	21-119	Received a request from pharmacology/toxicology reviewer. Already responded to this request on Dec. 10/99.
13-Jan-00	Regulatory Agency		Fax	21-119	Received a fax from FDA with divisions comments. Additional issues will be delt with after office level review.
17-Jan-00	QLT		Telephone Contact	21-119	Discussions with Lori Gorski re question 2a of the questions faxed to us on Jan. 13/00.
19-Jan-00	QLT		Telephone Contact	21-119	Lori Gorski called re question 2a and said that Carol Vincent was not satisfied with the information she sent to her and that we should put together a new submission.
28-Jan-00	QLT		Amendment	21-119	Submitted as amendment our response to the Agency's fax dated Jan. 13/00.
28-Jan-00	QLT		Fax	21-119	Faxed Lori a copy of the cover letter submitted today to FDA.She was also sent a DESK COPY of the submitted amendment.
28-Jan-00	QLT		Fax	21-119	Faxed Richard a copy of the cover letter, TOC, and device information submitted today to FDA.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 25

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
28-Jan-00	Regulatory Agency		Telephone Contact	21-119	Linda Carter called form FDA re financial disclosure information submitted.
31-Jan-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of our amendment submitted Jan. 28/00 in response to the Agency's fax dated Jan. 13/00.
31-Jan-00	QLT		Fax	21-119	Faxed Richard a copy of the cover letter Information Amendment - financial disclosure submitted to FDA today.
31-Jan-00	QLT		Fax	21-119	Faxed Lori a copy of the cover letter Information Amendment - financial disclosure submitted to FDA today.
31-Jan-00	QLT		Information Amendment	21-119	Submitted an Information Amendment - Financial Disclosure to FDA today.

Correspondence Table of Contents

Volume 26

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Feb-00	QLT		Information Amendment	21-119	Submitted draft promotional material (2 vols.) to Wiley as requested in a fax dated Jan. 13/00. An exact copy of what was sent to FDA (DDMAC).
01-Feb-00	QLT		Information Amendment	21-119	Submitted draft promotional material to FDA (DDMAC) (Vol. 1 of 2).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 27

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Feb-00	QLT		Information Amendment	21-119	Submitted draft promotional material to FDA (DDMAC) (Vol. 2 of 2).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 28

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
01-Feb-00	QLT		Information Amendment	21-119	Faxed Lori a copy of the cover letters submitted to FDA today.
01-Feb-00	Regulatory Agency		Telephone Contact	21-119	Lori Gorski called and requested another copy of the amendment submitted Jan. 28/00.
01-Feb-00	QLT		Information Amendment	21-119	Faxed Richard a copy of the cover letters submitted to FDA today.
01-Feb-00	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of information amendment - financial disclosure submitted Jan. 31/00.
02-Feb-00	Regulatory Agency		Telephone Contact	21-119	Warren called to clarify whether or not there should have been videos submitted or not.
02-Feb-00	Regulatory Agency		Telephone Contact	21-119	Irma Riviera called re FDA inspection.
02-Feb-00	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of Information Amendment - Draft Promotional Material submitted Feb. 01/00.
04-Feb-00	Regulatory Agency		Telephone Contact	21-119	Spoke with Lori re concern about our response to the clinical issue for approval. Set up teleconference with Wiley.
04-Feb-00	Regulatory Agency		Telephone Contact	21-119	Lori called re discussion with Wiley about labeling.
08-Feb-00	QLT		Fax	21-119	Faxed Richard a copy of the revised package insert submitted to FDA today.
08-Feb-00	QLT		Fax	21-119	Faxed Lori a copy of the revised package insert amendment submitted to FDA today.
08-Feb-00	QLT		Fax	21-119	Faxed Wiley a list of additional VISUDYNE data to be submitted to FDA and will be submitted by Feb. 18/00.
08-Feb-00	QLT		Information Amendment	21-119	Submitted to Wiley the revised package insert for VISUDYNE per discussion of Feb. 07/00.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 28

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
08-Feb-00	Regulatory Agency		Telephone Contact	21-119	Discussions between Tom Koestler and Wiley Chambers re approvable letter.
09-Feb-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of information amendment - revised package insert submitted Feb. 08/00.
10-Feb-00	QLT		Fax	21-119	Faxed Lori a copy of the 483 that was issued by the FDA at Nippon Fine Chemical Co. Ltd. on Feb. 09/00.
11-Feb-00	Regulatory Agency		Fax	21-119	Received a faxed copy of the approvable letter issued by FDA today.
15-Feb-00	QLT		Telephone Contact	21-119	Discussions with Lori re teleconference with Allan.
15-Feb-00	QLT		Telephone Contact	21-119	Discussion with Allan re information requested by Sept. 1/00.
15-Feb-00	Regulatory Agency		Fax	21-119	Allan requests further information on testing of raw material PRIX-DME required in the synthesis of the API verteporfin.
16-Feb-00	QLT		Fax	21-119	Faxed Lori a copy of the 483 that was issued to Sato Pharmaceutical Research Institute on Feb. 16/00.
17-Feb-00	Regulatory Agency		Telephone Contact	21-119	Voicemail from Warren Rumble re promotional material submission.
17-Feb-00	Regulatory Agency		Response	21-119	Response from DDMAC to our letter dated Feb. 01/00 requesting comments on the proposed promotional material for VISUDYNE. Given MACMIS ID #8690.
18-Feb-00	QLT		Fax	21-119	Faxed Richard a copy of the cover letter Information Amendment - Updated Safety and Efficacy Data submitted to FDA today.
18-Feb-00	QLT		Fax	21-119	Faxed Lori a copy of the cover letter Information Amendment - Updated Safety and Efficacy Data submitted to FDA today.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 28

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
18-Feb-00	QLT		Fax	21-119	Faxed Jonathan a copy of the cover letter Information Amendment - Updated Safety and Efficacy Data submitted to FDA today.

Correspondence Table of Contents

Volume 29

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
18-Feb-00	QLT		Information Amendment	21-119	Submitted an Information Amendment - Updated Safety and Efficacy Data in response to the approvable letter dated Feb. 11/00. (Vol. 1 of 5).

Correspondence Table of Contents

Volume 30

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
18-Feb-00	QLT		Information Amendment	21-119	Submitted an Information Amendment - Updated Safety and Efficacy Data in response to the approvable letter dated Feb. 11/00. (Vol. 2 of 5).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 31

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
18-Feb-00	QLT		Information Amendment	21-119	Submitted an Information Amendment - Updated Safety and Efficacy Data in response to the approvable letter dated Feb. 11/00. (Vol. 3 of 5).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 32

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
18-Feb-00	QLT		Information Amendment	21-119	Submitted an Information Amendment - Updated Safety and Efficacy Data in response to the approvable letter dated Feb. 11/00. (Vol. 4 of 5).

Correspondence Table of Contents

Volume 33

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
18-Feb-00	QLT		Information Amendment	21-119	Submitted an Information Amendment - Updated Safety and Efficacy Data in response to the approvable letter dated Feb. 11/00. (Vol. 5 of 5).

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 34

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
21-Feb-00	QLT		Fax	21-119	Faxed Richard a copy of the cover letter submitted to FDA today.
21-Feb-00	QLT		Fax	21-119	Faxed Jonathan a copy of the cover letter submitted to FDA today.
21-Feb-00	QLT		Fax	21-119	Faxed Lori a copy of the cover letter submitted to FDA today.
21-Feb-00	QLT		E-mail	21-119	Asking Richard whether or not it is acceptable to use the clinical lasers at the existing clinical centers for treatment of patients with commercial product.
21-Feb-00	QLT		Package Insert	21-119	Submitted two copies of the Revided VISUDYNE Package Insert to the attention of Warren Rumble.
22-Feb-00	QLT		Telephone Contact	21-119	Voicemail to Lori asking whether or not the micro reviewer had any issues.
22-Feb-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of information amendment - updated safety and efficacy data submitted Feb. 18/00.
22-Feb-00	Regulatory Agency		Telephone Contact	21-119	Allan called to say that he had no further questions.
24-Feb-00	QLT		General Correspondence	21-119	Sent Lori a desk copy of the updated safety and efficacy data submitted to FDA on Feb. 18/00 as well as a CD-ROM.
28-Feb-00	Regulatory Agency		Fax	21-119	A request from the pharmacology/toxicology reviewer.
01-Mar-00	Regulatory Agency	112	Acknowledgement	21-119	Acknowledgement of receipt of meeting minutes between QLT, Ciba Vision and FDA on June 23/99 submitted Feb. 29/00.
02-Mar-00	QLT		Amendment	21-119	Submitted our response to the agency's fax dated Feb. 28/00 concerning the final report for study TX-98008.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 34

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
03-Mar-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of amendment - our response to the agency's fax of Feb. 28/00 concerning the final report for study TX-98008 submitted Mar. 02/00.
03-Mar-00	QLT		General Correspondence	21-119	Submitted our response to DDMAC (MACMIS ID#8690) comments regarding draft promotional materials for VISUDYNE received Feb. 17/00.
03-Mar-00	Regulatory Agency		Telephone Contact	21-119	Warren Rumble called to set up teleconference to discuss letter.
03-Mar-00	QLT		General Correspondence	21-119	Information regarding the inspection of Sato Pharmaceutical Research Institute.
06-Mar-00	Regulatory Agency		Telephone Contact	21-119	Lori Gorski called re outcome of review meeting.
06-Mar-00	QLT		Information Amendment	21-119	Submitted our reply to the Chemistry Reviewer's fax dated Feb. 15/00.
06-Mar-00	QLT		Telephone Contact	21-119	Called Lori Gorski re chemistry commitment.
07-Mar-00	Regulatory Agency		E-mail	21-119	Reply to my request of aquiring the establishment inspection report of Nippon Fine Chemical Co. Ltd.
07-Mar-00	Regulatory Agency		Telephone Contact	21-119	Dr. Carreras called re patient related complaint.
07-Mar-00	QLT		Information Amendment	21-119	Submitted a copy of revised draft promotional material for VISUDYNE in response to DDMAC's letter dated Feb. 17/00. Also submitted two copies to Warren Rumble at DDMAC (MACMIS ID #8690).
07-Mar-00	QLT		E-mail	21-119	Asking for a copy of the establishment inspection report for Nippon Fine Chemical Co. Ltd.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 34

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
08-Mar-00	QLT		Telephone Contact	21-119	Spoke with Lori Gorski re drug and device applications - expecting the revisions to be postapproval.
08-Mar-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of information amendment - reply to chemistry reviewer fax of Feb. 15/00 submitted Mar. 06/00.
08-Mar-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of information amendment - revised draft promotional material in response to DDMAC's letter of Feb. 17/00 submitted Mar. 03/00 and Mar. 07/00.

Correspondence Table of Contents

Volume 35

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
14-Mar-00	QLT		Telephone Contact	21-119	Discussions with Wiley re status of VISUDYNE review.
15-Mar-00	QLT		Telephone Contact	21-119	Called Lori to set up teleconference to discuss the outcome of the DSMC meeting for the 2 year TAP and 1 year VIP data.
16-Mar-00	Regulatory Agency		Fax	21-119	Comments from DDMAC (MACMIS ID #8690) in response to our submission (scripts for ophthalmology guides and patient-directed CD-ROM) dated March 7/00.
16-Mar-00	Regulatory Agency		Response	21-119	Comments from DDMAC (MACMIS ID #8690) in response to our promotional materials submission (revised sales aid and journal ad) dated March 3/00.
16-Mar-00	QLT		Telephone Contact	21-119	Confirmation of teleconference Friday Mar.24 and FDA okay with draft press release.
20-Mar-00	Regulatory Agency		E-mail	21-119	Lori's response to my question re acceptable to revise the Patent Certification information during review process.
21-Mar-00	QLT		Telephone Contact	21-119	Asking Richard about the acceptability of using the clinical lasers at the existing centers for treatment of patients with commercial product.
23-Mar-00	QLT		Fax	21-119	Faxed Lori the information (contents of press release) that is to be discussed with Dr. Chambers tomorrow by telephone.
24-Mar-00	Regulatory Agency		Fax	21-119	Draft labeling information from FDA.
24-Mar-00	QLT		Telephone Contact	21-119	Teleconference with FDA re press release for 2-year TAP data and 1-year VIP data.
24-Mar-00	QLT		Information Amendment	21-119	Submitted three copies of an information amendment - Revised Patent Information.
24-Mar-00	QLT		Information Amendment	21-119	Submitted three copies of two information amendments - Draft Press Release and the 1-year Results of the VIP Study.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 35

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
26-Mar-00	Regulatory Agency		Fax	21-119	FDA audit of MEEI preclinical studies of verteporfin that took place Feb. 16/00 - Mar. 03/00.
27-Mar-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of two information amendments - (1)draft press release and the 1-year results of the VIP study and (2) revised patent information, both submitted Mar. 24/00.
27-Mar-00	QLT		Fax	21-119	Faxed Wiley the draft press release of Monday, March 27, 2000 @ 12:30 E.S.T. (released March 28, 2000).
27-Mar-00	Regulatory Agency		Telephone Contact	21-119	Carol asked if EtO was used to sterilize any of the components.
28-Mar-00	Regulatory Agency		Telephone Contact	21-119	Lori called re the 1 year resluts from the VIP study.
30-Mar-00	Regulatory Agency		Telephone Contact	21-119	Carol needs additional validation information.
30-Mar-00	QLT		Fax	21-119	Faxed Carol Vincent the reply to the information she requested.
30-Mar-00	QLT		Telephone Contact	21-119	Called Lori to let her know that Carol Vincent is askinf for more Microbiology information.
31-Mar-00	QLT		Amendment	21-119	Submitted the micribology information requested by Carol Vincent on March 30/00.
31-Mar-00	Regulatory Agency		Telephone Contact	21-119	Lori called to discuss the review, labelling, approval, press release, etc.
03-Apr-00	QLT		Information Amendment	21-119	Submitted information amendment - Final Draft Labelling in acceptance of the draft labelling received from FDA by fax on Mar. 24/00.
03-Apr-00	Regulatory Agency		Telephone Contact	21-119	Lori called re address clarification.
03-Apr-00	QLT		Information Amendment	21-119	Submitted an information amendment - Request for Exclusivity for the molecular entity verteporfin for injection.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 35

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
03-Apr-00	Regulatory Agency		Telephone Contact	21-119	Lori called to remind us of the need to submit the letter of label agreement.
03-Apr-00	Regulatory Agency		Telephone Contact	21-119	Lori called to ask for the request to have exclusivity.
03-Apr-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of microbiology amendment submitted March 30/00.

Correspondence Table of Contents

Volume 36

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
04-Apr-00	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of two information amendments; (1) Request for Exclusivity and (2) Final Draft Labeling, both submitted April 03/00
05-Apr-00	QLT		Information Amendment	21-119	Submitted information amendment - draft promotional material. Two copies were also submitted to DDMAC.
05-Apr-00	QLT		Telephone Contact	21-119	I called Lori to discuss the clinical and microbiology reviews.
05-Apr-00	QLT		General Correspondence	21-119	Submitted two copies of four new draft promotional pieces for VISUDYNE to DDMAC (MACMIS ID #8690) for comments.
06-Apr-00	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of Information amendment - draft promotional material submitted Apr. 05/00.
07-Apr-00	Regulatory Agency		Fax	21-119	Received by fax from FDA a copy of the revisions to the final draft labeling submitted today.
07-Apr-00	QLT		Telephone Contact	21-119	I called Lori to give her the hotel information for Larry and I in case anything came up in the labeling.
07-Apr-00	QLT		E-mail	21-119	Sent Lori via email the final draft package insert.
07-Apr-00	QLT		Information Amendment	21-119	Submitted information amendment - Final Draft Labeling per the revisions received from FDA today.
07-Apr-00	Regulatory Agency		Telephone Contact	21-119	Lori called to go through edits of the final draft labeling.
10-Apr-00	Regulatory Agency		Telephone Contact	21-119	Lori called re correction in labelling submission dated Apr. 07/00.
10-Apr-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of Information Amendment - Final Draft Labeling submitted Apr. 07/00.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 36

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
12-Apr-00	QLT		Fax	21-119	Faxed Lori a copy of the "approval" press release for April 13/00.
12-Apr-00	QLT		Fax	21-119	Faxed Raye a copy of the "approval" press release for Apr. 13/00.
12-Apr-00	Regulatory Agency		Approval Letter	21-119	VISUDYNE IS APPROVED!!!!! Approval letter from FDA.
12-Apr-00	QLT		Telephone Contact	21-119	Discussions with Wiley re issues related to approval and beyond.
13-Apr-00	QLT		Telephone Contact	21-119	Asking Lori where to send the market vial.
13-Apr-00	Regulatory Agency		Telephone Contact	21-119	Rae called re our press release. She faxed us her press release.
13-Apr-00	QLT		Telephone Contact	21-119	Spoke with Lori today re approval letter.
13-Apr-00	Regulatory Agency		Fax	21-119	Received a faxed copy of FDA's press release for the approval of VISUDYNE.
18-Apr-00	Regulatory Agency		General Correspondence	21-119	Response from DDMAC (MACMIS ID #8690) re our submission dated April 05/00 regarding three direct-to-consumer advertisements.
20-Apr-00	QLT		General Correspondence	21-119	Submitted a Market Package of Drug Product including the drug product, vial, unit carton, vial label and package insert used for launch.
25-Apr-00	QLT		Telephone Contact	21-119	Spoke with Warren Rumble re 2 year data.
26-Apr-00	Regulatory Agency		Fax	21-119	Acknowledgement of receipt of Market Package of Drug Product Submission dated April 20/00.

Correspondence Table of Contents

Volume 37

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
02-May-00	QLT		General Correspondence	21-119	Submitted Draft Promotional Materials - patient guide video and physicians preparation and delivery video.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 38

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
04-May-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of draft promotional materials - patient guide video and preparation and delivery video for VISUDYNE submitted May 02/00.
08-May-00	QLT		General Correspondence	21-119	Submitted the final printed promotional materials; journal ad and index card.
09-May-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of final printed promotional material submitted May 8/00.
11-May-00	QLT		Telephone Contact	21-119	Spoke with FDA today re international birthdate for reporting purposes.
11-May-00	QLT		General Correspondence	21-119	Submitted Draft Promotional Materials - two videos and four posters.

Note: Highlighted correspondence is not finalized and not yet filed in the binder.

Correspondence Table of Contents

Volume 39

05-Jun-00

Date	Initiator	ID #	Type	Submission Reference Number:	Description
15-May-00	Regulatory Agency		Acknowledgement	21-119	Acknowledgement of receipt of draft promotional materials submitted May 11/00.
15-May-00	Regulatory Agency		Telephone Contact	21-119	Warren called to provide unofficial comments on the Zeiss and Coherent videos and posters submission.
15-May-00	Regulatory Agency		Deficiency Letter	21-119	Comments from DDMAC (MACMIS ID #8690) on draft promotional materials (patient guide video and physicians guide video) submitted May 02/00.
16-May-00	Regulatory Agency		Deficiency Letter	21-119	Response from DDMAC (MACMIS ID #8690) on draft promotional material - two videos and four posters submitted May 11/00.

SKIN CANCER IND

Correspondence

Correspondence Table of Contents

Volume 1a

16-Feb-04

Date	Initiator	ID #	Type	Submission Reference Number:	Description
25-Apr-90	QLT		General Correspondence	37,129	Submitted our Pre-IND Meeting Package scheduled for May 4, 1990 at 1:00pm.

Correspondence Table of Contents

Volume 1-8

16-Feb-04

Date	Initiator	ID #	Type	Submission Reference Number:	Description
12-Jun-91	QLT	000	General Correspondence	37,129	Submitted a Notice of Clinical Investigational Exemption for a New Drug for Benzoporphyrin Deriviative Monoacid Ring A (BPD-MA), a photosensitizing agent for the treatment of cutaneous malignancies.

Correspondence Table of Contents

Volume 1

16-Feb-04

Date	Initiator	ID #	Type	Submission Reference Number:	Description
25-Jun-91	Regulatory Agency		Acknowledgement	37,129	Acknowledge receipt of IND application. IND # assigned 37,129. Date of receipt 06-17-91.
11-Jul-91	Regulatory Agency		General Correspondence	37,129	BPD-IND FDA Contact with Dr. Blumenstein; safety questions re bulk active and finished product.
18-Jul-91	Regulatory Agency		General Correspondence	37,129	FDA's recommended changes for approval to start Phase I study.
26-Jul-91	QLT	001	Protocol Amendment	37,129	Change in Protocol.
06-Aug-91	Regulatory Agency		General Correspondence	37,129	FDA Ocular recommendations.
27-Aug-91	Regulatory Agency		General Correspondence	37,129	Comments/corrections to BPD FDA safety questions re: bulk active and finished product.
28-Aug-91	Regulatory Agency		Fax	37,129	FDA fax stating the editorial rewrites for protocol amendment.
30-Sep-91	QLT	002	General Correspondence	37,129	Response to FDA Request for Information - Chemistry.
03-Oct-91	QLT	003	Protocol Amendment	37,129	Change in Protocol (Amendment #2), New Investigator, Dr. Stuart Salasche.
14-Nov-91	QLT	004	General Correspondence	37,129	Follow up to confirm our request to initiate a standard Phase I study.
29-Jan-92	QLT	005	General Correspondence	37,129	Single Patient Exception - Patient #1.
29-Jan-92	QLT	005	General Correspondence	37,129	SPE Patient #1.
24-Feb-92	QLT	006	Protocol Amendment	37,129	

Correspondence Table of Contents

Volume 2

16-Feb-04

Date	Initiator	ID #	Type	Submission Reference Number:	Description
03-Mar-92	Regulatory Agency		General Correspondence	37,129	Letter from R. Anderson re non-invasive techniques.
04-Mar-92	Regulatory Agency		General Correspondence	37,129	Draft of fax from G. Dukart to R. Anderson in response to R. Andersons's letter re non-invasive techniques.
26-Mar-92	QLT	007	Protocol Amendment	37,129	Protocol Amendment #4 - Clinical.
30-Apr-92	Regulatory Agency		General Correspondence	37,129	Policy Regarding Documentation of Investigator Qualifications.
01-May-92	QLT	008	Information Amendment	37,129	Adverse Events.
03-Jun-92	QLT	009	Safety Report	37,129	Initial Report
10-Jul-92	QLT	010	Protocol Amendment	37,129	New Subinvestigator: L. Hruza/Follow up to Written Report.
14-Jul-92	QLT		General Correspondence	37,129	Letter informing that a patient died on 07-10-92.
10-Aug-92	QLT	011	Annual Report	37,129	Annual Report, June 91 to June 92.